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SOIL SCIENCE

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FIELD METHOD FOR LIME REQUIREMENT OF SOILS[1]

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Illinois Agricultural Experiment Station

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The determination of the base-exchange capacities of the major Illinois soil types has brought out the fact that these soils vary greatly in this respect (1). The variation is so great that it makes it impossible to make an entirely satisfactory lime requirement recommendation based on any method which measures either directly or indirectly only the active hydrogen ions in the soil.

Since in any well-drained soil in a humid region the hydrogen-ion concentration may be considered to be a resultant of the degree of saturation of the soil base-exchange complexes with bases, it follows that the same degree of saturation of these complexes with bases in two different soils may give approximately the same hydrogen-ion concentration, and yet the amount of bases present in each and also the amount of bases necessary to saturate completely these soils may vary considerably. The two extremes in Illinois are represented on the one hand by sandy loams having a base-exchange capacity as low as 3.4 m.e.,[3] and on the other hand by the clay loams having base-exchange capacities as high as 37.3 m.e. Starting with any given degree of unsaturation, the clay loams would require approximately 10 times as much base to saturate them fully as the sandy loams require. To illustrate this, two samples have been selected from over 250 soils in which had been determined, first, the pH by the hydrogen electrode method; second, the total replaceable bases by a titration method (2); and, third, the base-exchange capacity by a modification of Kelley's method (6) in which $CaCl_2$ instead of $NH_4C_2H_3O_2$ was used as the replacing agent. From these data the degree of saturation was calculated by dividing the milli-equivalents of replaceable bases present by the base-exchange capacity and multiplying by 100.

The first soil of the two illustrative examples is a sample of brown silt loam, with a base-exchange capacity of 21.5 m.e. and 11.5 m.e. of replaceable bases present. The second is a sample of light gray silt loam on tight clay with a

[1] Contribution from the division of soil fertility, department of agronomy, University of Illinois, Urbana, Illinois. Published with the approval of the director of the experiment station.

[2] Associate in soil survey analysis and chief in soil technology, respectively.

[3] The abbreviation m.e. represents milligram-equivalents in 100 gm. of soil. For those who are accustomed to think in terms of field limestone requirements, it may be stated that 1 m.e. computed to $CaCO_3$ is equivalent to 1,000 pounds of $CaCO_3$ an acre of 2,000,000 pounds of soil.

base-exchange capacity of 10.9 m.e. and 5.7 m.e. of replaceable bases present. To bring the brown silt loam up to 80 per cent of saturation[4] will require 5,700 pounds of $CaCO_3$ an acre (2,000,000 pounds soil), whereas the light gray silt loam on tight clay will require only 3,000 pounds. The degree of saturation is 53 per cent in both cases, the pH of both soils is 5.4, and the colors produced by the thiocyanate test are almost identical. The base-exchange capacity, the total replaceable bases, and the calculated lime requirements are almost exactly double for the one soil as compared with the other, yet the degree of saturation and the pH are the same.

As has been stated, these two samples were selected from more than 250, as ideal examples to show the non-conformity of the pH values with calculated

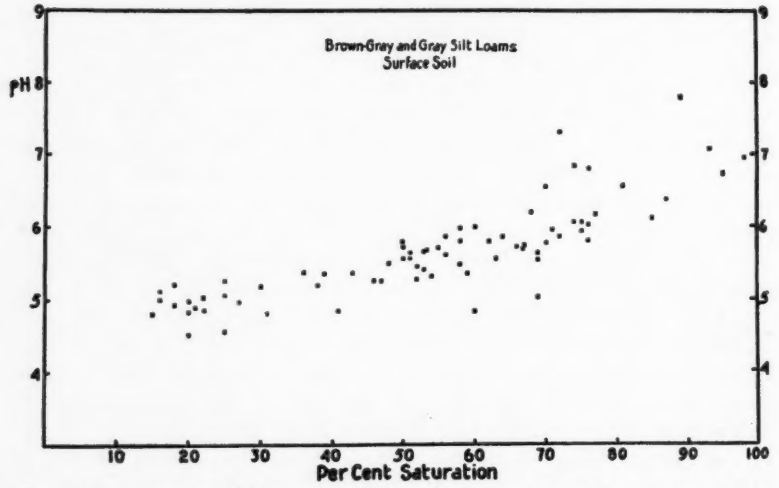

FIG. 1.—RELATION OF pH VALUES AND DEGREE OF BASE-SATURATION OF THE BASE-EXCHANGE CAPACITY IN 74 SAMPLES OF SURFACE SOIL

These samples represent 16 soil types, and base-exchange capacities ranging from 7 to 17 m.e.

lime requirement. A consideration of a large number of results gives a more accurate conception of the relation between these two values. Figure 1 shows the pH values and percentage of saturation of some surface (unlimed) soils, brown-gray silt loams, and gray silt loams, plotted against each other. The base-exchange capacities of these soils range from 7 to 17 m.e. These values as plotted form a curve. The breadth of the curve, however, indicates either

[4] Investigations in this laboratory have shown that satisfactory growth of sweet clover is obtained on many widely different soil types when the degree of saturation with replaceable bases is 70 to 80 per cent. The limestone necessary to raise the degree of saturation to the upper figure, 80 per cent, has therefore been chosen as representing the lime requirement of the soil.

that our present methods are not sufficiently refined or else that other factors which influence this relation are not being considered. A difference in the nature of the base-exchange complexes in the different soil types, or the presence of Ca or Mg salts of organic acids soluble in the salt leaching solution would, if present, tend to broaden the curve. It is found further that this curve is relatively flat below 60 per cent saturation but rises fairly abruptly at this point and at the same time becomes somewhat broader. Thus, the correlation between pH and degree of saturation of the base-exchange capacity is better in those soils which are more than 60 per cent saturated, than in less saturated soils.

The heavier silt loams and the clay loams have been omitted from figure 1 because certain samples among them show such a wide variation from the curve that it is evident that the aforementioned method of calculating the degree of saturation cannot be entirely relied upon for all soils. These are poorly drained types high in organic matter. They give an apparently high degree of saturation accompanied by a fairly low pH. Further studies on these soils have shown that they contain more acid than can be accounted for by the difference between the base exchange capacity and the replaceable bases present, as measured by the aforementioned methods.

From these relationships, it is obvious that there are two objections to the use of the hydrogen-ion concentration or any value depending upon it as a basis for limestone applications. One objection is the absence of a sufficiently close correlation between pH values and degree of saturation of the base-exchange capacity in soils which are less than 60 per cent saturated. The other objection is that the base-exchange capacities of soils vary greatly and unless this value is known in the case of an individual soil sample, the pH value gives no clue as to the amount of limestone needed to bring the degree of saturation up to any desired level. Morgan (10), in discussing pH and lime requirement, gives a review of the literature on this subject which indicates that a relation exists only on soils of the same type or closely related types. Where soils of such similar types are used the base-exchange capacities are very likely to be similar (1), a situation which would partially overcome the second objection stated, i.e., that the base-exchange capacities may vary greatly.

The measurement of all the acid present, or of all the base which the soil will absorb, would give a value entirely too high for a limestone recommendation. It is well known that the pH of the endpoint in titrating a weak acid against a relatively strong base is considerably above 6.5 to 7, which may be considered an endpoint for liming as far as satisfactory sweet clover growth is concerned. Figure 1 shows that this pH range in unlimed soils occurs at approximately 80 per cent saturation.

The average value for the limed plots on seven Illinois experiment fields on widely varying soil types which grow excellent sweet clover is 81 per cent saturation. Although sweet clover[5] will grow at pH values below 6.5 or at a

[5] Sweet clover has been chosen as a reference crop partly because of its high sensitivity to acid soil conditions, and partly because of the importance of conditioning soils to grow this crop for soil improvement.

degree of saturation below 80 per cent, it has been found in greenhouse pot experiments[6] that the maximum crop was obtained only where the degree of saturation with bases had been brought to between 70 and 80 per cent. If a satisfactory condition for sweet clover growth can be obtained by increasing the degree of saturation to 80 per cent, one is hardly justified in making a limestone recommendation based on the total acid present, since with soils of high base capacity this extra 20 per cent would mean an increase of 3 or 4 tons of fine limestone, or 4 or 5 tons of screenings.

As a result of these conclusions, it was thought desirable to devise a method which would measure approximately the amount of limestone necessary to increase the degree of saturation of the soil with bases to 80 per cent. Such a method would be of practical importance as a means of measuring lime requirement. A method has been worked out in this laboratory using for its calibration the numerous soil samples on which the base-exchange capacity and degree of saturation have already been determined. This method is based on the following reasoning.

When a sample of acid soil is shaken in a salt solution, the base of the salt replaces part of the replaceable hydrogen. The resulting equilibrium is expressed by the following equilibrium equation:

$$KCl + H\,X \rightleftharpoons HCl + K\,X$$

where X represents the colloidal soil complex capable of the surface absorption of H ions and other cations. Now, if a small amount of base or base reacting salt such as Na_2CO_3 be added to the solution, the HCl will be neutralized until all the added base is used up and a new equilibrium will be established during which more K ions will replace more H ions on the colloidal surface. Since a part of the total amount of acid present will have been neutralized, the resulting new equilibrium will be one in which a smaller number of hydrogen ions exists in the solution, and consequently, the pH of the solution will be higher. As more and more base is added, the pH will continue to rise. Now if to any given soil whose base exchange capacity and amount of replaceable bases are known, just enough base is added to increase the degree of saturation to 80 per cent, the resulting solution should have a certain pH which will be for all practical purposes very similar for any soils so treated. Peats, mucks, and similar soils are not included in this discussion, although it is believed that the method as finally worked out can be used on them.

It was reasoned that if an indicator could be found whose color change comes at about this pH, one could then take a soil whose degree of saturation and base-exchange capacity are unknown, shake separate portions of it with a salt solution with varying known amounts of base, and determine what amount of base is necessary to bring about the desired change in color of the indicator.

[6] E. E. DeTurk and R. H. Bray, unpublished work.

Experiments have shown that when KCl is the salt used, and Na_2CO_3 is the base used, this endpoint can be determined on soils of varying base-exchange capacities and degrees of saturation by the use of bromthymol blue. The intermediate green to green-blue color of this indicator gives the endpoint for this determination. The procedure used was adopted after numerous trials with various salts, different indicators, and varying amounts of water.

This determination is, in effect, a titration method, but differs from the usual recommended methods [see review of methods by Clark and Collins (4)], in that the endpoint of the titration was chosen as a result of its agreement with an 80 per cent degree of saturation rather than selecting, for example, a pH of 7 for the endpoint because it represents neutrality.

In developing the method, the calibration was effected by using numerous soil samples whose base-exchange capacity, total replaceable bases, and pH were known. The soils used represented a wide range of types from black clay loams through brown silt loams to light sandy loams. A few samples of the darker soils whose pH did not seem to agree with the degree of saturation within the limits of the experimental curve were not used in the calibration, although the results on them obtained with the method as finally adopted have been included in table 1. This table gives for comparison the amounts of limestone necessary to reach an 80 per cent degree of saturation as calculated from previously determined base-exchange data and the amounts as found by the titration method. It will be noticed that the titration method has an advantage over the base-exchange method, since by the former a definite though small lime requirement is given for those soils whose calculated degree of saturation is high and yet which have a low pH. A low pH indicates unsaturation, even though it is not a quantitative measurement of the amount of limestone needed.

The general agreement between the lime requirement as calculated from the base-exchange data and that found by the titration method is fairly good except for the soils already mentioned as being subject to error in the base-exchange data. In fact, Chapman and Kelley (3) conclude that neither the replaceable base methods nor the base-exchange capacity determinations yield highly exact results, so that a difference of 1 m.e. corresponding to 1,000 pounds of $CaCO_3$ per acre would not be accounted a large error in any soil. The soils have been arranged in table 1 in order of increasing calculated requirements and arbitrarily separated into groups. For any one group the average lime requirement, as computed from the base-exchange data, is approximately the same as that found by the titration method. Excluding the first three groups containing neutral soils and most of those soils subject to error by the base-exchange method, there are only 5 cases in 61 in which the variation between the two methods is over 1 ton. Since the results of the titration method are likely to vary either above or below the calculated amount, it is considered that this method measures approximately the amount of bases necessary to raise the degree of saturation to 80 per cent.

TABLE 1

Comparison of the amount of limestone in pounds per acre necessary to raise the degree of saturation to 80 per cent as determined by calculation from base exchange data and by the direct determination by the Na_2CO_3 method

LABORATORY NUMBER	TYPE NUMBER*	BASES REQUIRED CALCULATED	BASES REQUIRED BY Na_2CO_3 METHOD	pH	THIOCYANATE†
		pounds	*pounds*		
12059	17	0	0	7.79	0
13058	14	0	0	7.07	0
11941	18	0	0	6.95	1
11976	43	0	0	6.85	0
13012	14	0	0	6.64	0
12096	56	0	0	6.60	0
	Average.....	0	0		
11931	18	0	430	6.72	0
11936	18	0	1,290	6.56	0
11914	41	0	860	6.43	0
11982	65	0	860	6.48	0
	Average.....	0	860		
12053	66	0	430	6.99	1
12018	66	0	430	6.63	1
11964	43	0	860	6.31	2
12029	66	0	1,290	6.28	2
11988	41	0	1,290	6.15	2
11293	43	0	430	6.11	3
12024	66	0	1,720	6.25	4
11958	43	0	1,290	5.88	4
11995	41	0	1,290	5.86	4
11970	43	0	1,290	5.64	4
11898	41	0	1,290	5.45	4
11908	41	400	860	6.16	2
12005	41	600	2,580	5.66	4
	Average.....	77	1,158		
11300	127	1,000	1,290	6.07	2
11919	41	1,300	1,720	6.07	1
11314	46	1,300	2,580	5.95	3
12035	59	1,400	1,720	5.90	2
S6393	41	1,500	3,010	5.52	3
11892	41	1,500	3,440	5.29	5
11925	41	1,500	1,290	5.93	3
11307	127	1,500	1,720	5.87	3
12012	41	1,500	1,290	6.18	3
11807	11	1,700	2,150	5.04	4
11342	46	1,800	2,150	5.76	2
12069	18	1,800	3,010	5.84	3
	Average.....	1,483	2,114		

* Illinois soil survey type number.

† Thiocyanate readings reported on scale of 0 = no color; 5 = dark red.

TABLE 1—*Continued*

LABORATORY NUMBER	TYPE NUMBER*	BASES REQUIRED CALCULATED	BASES REQUIRED BY Na_2CO_3 METHOD	pH	THIOCYANATE†
		pounds	*pounds*		
11825	2	2,100	3,010	4.83	5
11180	112	2,100	2,580	5.69	4
13030	14	2,200	1,720	5.95	3
11335	124	2,300	2,580	5.73	5
13029	14	2,300	1,290	5.78	3
11876	14	2,400	1,290	5.97	4
11229	11	2,600	2,550	5.37	4
13036	14	2,600	2,150	5.56	4
13043	14	2,700	1,720	5.66	3
13037	14	2,800	1,720	5.52	4
11864	13	2,900	2,150	5.72	4
11222	12	2,900	3,010	5.37	3
11870	13	2,900	2,150	5.48	4
11257	11	3,000	3,870	5.41	5
	Average.....	2,557	2,271		
13024	14	3,200	2,150	5.26	4
11857	12	3,500	2,580	5.49	2
11250	11	3,500	5,160	5.20	4
13000	14	3,600	3,010	5.56	3
11349	127	3,800	2,150	5.79	3
11286	127	3,900	3,870	5.51	4
13070	14	4,000	2,510	5.25	3
	Average.....	3,643	3,061		
11145	113	4,200	3,870	5.32	3
13071	14	4,200	3,010	4.76	4
11243	12	4,400	5,590	5.20	4
11272	12	4,400	3,440	5.26	3
13044	14	4,400	4,300	5.14	4
11882	21	4,500	4,300	5.05	5
S6391	41	4,500	5,160	5.15	3
11903	41	4,700	2,510	5.50	4
11215	11	4,800	4,730	5.11	4
11831	2	4,800	4,730	4.82	5
11852	12 (subsoil)	4,900	4,400	4.74	5
	Average.....	4,527	4,185		
11321	127	5,000	5,160	5.79	2
11279	12	5,200	4,300	4.88	5
S6396	41	5,200	4,370	5.16	3
11236	12	5,300	6,450	5.00	4
S6399	41	5,300	3,440	5.45	3
11328	127	5,500	4,300	5.28	3
11851	12	5,950	5,600	4.51	5
	Average.....	5,350	4,803		

TABLE 1—*Concluded*

LABORATORY NUMBER	TYPE NUMBER*	BASES REQUIRED CALCULATED	BASES REQUIRED BY Na_2CO_3 METHOD	pH	THIOCYANATE†
		pounds	*pounds*		
11851	12	6,100	6,020	4.51	5
11853	12 (subsoil)	6,600	6,400	5.17	5
12089	56	7,600	4,300	5.56	3
11801	1	7,800	5,590	4.86	4
11813	1	8,200	5,160	5.00	5
11880	21	8,900	9,460	5.17	3
11837	3	10,800	9,460	4.80	5
11856	12 (subsoil)	11,800	13,200	5.06	5
11854	12 (subsoil)	13,500	16,800	5.13	5
11855	12 (subsoil)	14,600	16,400	5.17	5
	Average.....	9,590	9,279		

In the foregoing discussion the term "lime requirement" has been used to designate the amount of $CaCO_3$ necessary to raise the degree of saturation with bases to 80 per cent. Other factors which may influence the efficiency of the limestone added in bringing about this degree of saturation must also be considered.

One of these factors is the fineness of the limestone. Coarse particles which remain in the soil for years may serve as alkaline centers, yet neutralize only a small part of the surrounding soil. It may be possible by adding enough of these coarse particles to furnish sufficient alkaline centers to promote sweet clover growth. Pot culture experiments at this station represented by the following data indicate that where limestone which passes through an 8-mesh but over a 10-mesh sieve is used, more than twice as much is required for good sweet clover growth as of fine limestone (through 100 mesh).

RATE PER ACRE 2,000,000 POUNDS SOIL	CONDITION OF SOIL AT END OF 2 YEARS	SWEET CLOVER YIELDS	
		2 years after liming	5 years after liming
		gm.	*gm.*
7,000 pound 100-mesh stone.....	pH 6.63 74 per cent saturated	20.1	17.2
10,000 pound 8–10-mesh stone.....	pH 5.56 44 per cent saturated	12.1	15.1
2,000 pound 100-mesh stone.....	pH 5.57 38 per cent saturated	6.6	0
No limestone.....	pH 4.82 29 per cent saturated	0	0

The soil receiving 2,000 pounds of fine, high-calcium stone grew a small crop of sweet clover the first time only, whereas the soil receiving 10,000 pounds of coarse stone continued to grow sweet clover for more than 5 years but with a lower yield than was obtained on the soil receiving 7,000 pounds of fine material.

It is evident that the coarse particles, besides slowly raising the degree of

saturation, are serving as alkaline centers which are used by the sweet clover. The growth of sweet clover, however, is not satisfactory and a system of liming depending on these large particles alone would require enormous amounts of such limestone on strongly acid soils. Therefore, the effectiveness of the limestone in raising the degree of saturation should be the main consideration in evaluating limestones of varying degrees of fineness. Average limestone screenings, as used in Illinois, were found, when applied to a highly acid gray silt loam soil, to be from 75 to 80 per cent effective over a 5-year period as compared to finely pulverized limestone. The ineffective portion consisted, in the main, of coarse particles recoverable after the 5 years as $CaCO_3$.

Another factor in considering the amount of limestone required to hold good over a period of years is the rate of leaching. Once a high degree of saturation is reached, any excess of fine $CaCO_3$ is soluble in the slightly carbonated rain water and is fairly rapidly taken down below the plowed depth. However, if no excess $CaCO_3$ is added and all the Ca applied is taken up in the replaceable form, the loss is slower compared to the loss incurred in trying to maintain a high degree of saturation with excess limestone present. That the rate of loss of replaceable calcium increases as the degree of saturation increases was indicated in this laboratory by leaching soils of different degrees of saturation with carbonated water. It is considered by the writers to be uneconomical as well as unnecessary to try to maintain a degree of saturation above 80 per cent.

In the pot culture work referred to in the foregoing, an average loss of 23 per cent was observed, part of which may have been due to leaching and part to another factor to be considered next.

Crowther (5) mentions that the change in pH which occurs in the field due to liming is less than that shown in the laboratory. Pierre and Worley (11) in estimating the amount of lime necessary to bring the soil to definite pH values by means of the buffer method and the determination of exchange hydrogen, finds that a factor of 1.5 is necessary to correct for inefficiency in the action of the lime compared to the laboratory methods where the efficiency is measured by adding known amounts of lime to small glasses full of moist soil and allowing them to stand for a relatively short period. MacIntire and Sanders (9) found a loss of lime approximating 16 per cent which could not be accounted for in the replaceable or carbonate forms or in the drainage waters. They think it indicates a combination "with soil components other than the exchange complex."

Whatever the cause of this loss, it must be charged against the efficiency of the limestone in raising the degree of saturation. MacIntire and Sanders' total loss, including the drainage loss, amounted to about 21 per cent of the amount added. This compares favorably with the loss of about 23 per cent of the amount decomposed as found by the writers. Although one figure is based on amounts added and the other on amounts decomposed, they can be compared since in both cases practically all of the limestone was decomposed except in a few instances where large particles were used.

Values have been computed from Lunt's (8) work on total and replaceable calcium in soils from eight Illinois experiment fields, which indicate that this possible "fixation" may be very slight in long-time experiments where large amounts of calcium carbonate have been added. Only one field, Ewing, out of the eight fields studied, shows any appreciable increase in total calcium on the limed plots which is not accounted for in the replaceable or carbonate forms. The limestone additions have in some cases doubled the total calcium content of the soil and the comparison of total amounts in adjacent limed and unlimed plots should be fairly significant, subject only to variation in the total amounts originally present.

In the field plots, however, the total amount of calcium added as limestone through a period of years cannot be accounted for in the surface, a large percentage having been lost from the plowed depth. In these field experiments the limestone was added in excess of the amount required, as high as 20,000 pounds per acre having been added in some cases over a period of years to soils with base-exchange capacities equivalent to 10,000 pounds. The statement made earlier in this paper that loss due to leaching is greater where excess $CaCO_3$ is present and the base-exchange complexes are highly saturated is borne out by these heavy losses on highly limed plots and also by the relatively small losses of replaceable bases which have been suffered by acid check plots over periods up to 25 years.

In using this titration method, the aforementioned factors should be considered and corrections made for them. It has been decided that, for the present, the titration value should be multiplied by 1.67. This gives the limestone screenings used in this state an efficiency of 60 per cent based on 100 per cent $CaCO_3$ equivalent. Twenty per cent of the reduction is assumed to be due to the coarseness factor and 20 per cent is considered a safety factor, which includes any relatively early loss of the available forms by leaching, as well as any possible "fixation." In the case of finely ground limestone (all passing a 35-mesh Tyler sieve), the correction for coarseness is omitted and the efficiency factor is, therefore, 1.25.

THE LABORATORY METHOD

Procedure.—To each of a series of 5-gm. samples of 10-mesh, air-dry soil (not ground) in test tubes add 1 gm. KCl and 25 cc. distilled water. To the first, add standard Na_2CO_3 solution equivalent to 0.0062 gm. $Na_2CO_3 \cdot H_2O$. This is equivalent to 1 ton of $CaCO_3$ to an acre, 2,000,000 pounds of soil. To the others add two, three, and four times this amount, respectively. Half-ton intervals instead of 1-ton may be used if desired. Shake vigorously about 25 times and let settle. Then add three drops of bromthymol blue to each tube and agitate very gently, just enough to mix the indicator with the upper half-inch of solution. The first tube showing a green to greenish blue color is considered as having had sufficient base and the corresponding amount of $CaCO_3$ is taken as the lime requirement. When half-ton intervals are used,

the color in the tube just below the one giving the endpoint will usually be yellow, and the one just above will be blue. A yellow-green color should be taken as the endpoint only in case the tube having the next larger amount of Na_2CO_3 shows a blue. If the colors of two adjacent tubes are yellow and blue, respectively, the endpoint may be taken as half way between these two. By making a preliminary thiocyanate test, or one of the available indicator tests, it is usually possible to reach the endpoint with only three trials, using, for example, 1-, 2-, and 3-ton equivalents for slightly acid soils and 3-, 4-, and 5-ton equivalents for those apparently more acid. In the original trials reported in table 1, an amount of Na_2CO_3 solution equivalent to 430 pounds of $CaCO_3$ an acre, or a multiple, was used in the different trials. This interval, however, is too small to give an endpoint sharp enough for practical use.

Although this method as outlined could be made portable for field use, the possible difficulty of maintaining properly standardized Na_2CO_3 solution and calibrated medicine droppers in lieu of burettes led to the following modification which is designed for use in the field.

THE FIELD METHOD

Materials.—(*a*) $Na_2CO_3 \cdot H_2O$-KCl tablets. Mono-hydrated sodium carbonate, $Na_2CO_3 \cdot H_2O$, and KCl are ground together and compressed into tablets such that each tablet contains 0.5 gm. KCl and 0.0062 gm. $Na_2CO_3 \cdot H_2O$; (*b*) bottle of distilled water; (*c*) bromthymol blue in dropping bottle; (*d*) mortar and pestle for crushing soil; (*e*) spatula; (*f*) special 5-gm. measuring cup (5 cc. capacity); (*g*) test tubes, 3/4 inches by 6 inch and test tube rack; and (*h*) graduated cylinder, 25 cc.

Procedure.—Measure three to four 5-gram samples into separate test tubes. The measuring cup is filled and slightly heaped by pouring the crushed, but unground soil, into it with the spatula, avoiding any shaking or jarring of the cup since this would cause packing. When full, strike off level with the edge of the spatula. Except for sands, mucks, and peats, these samples will vary only slightly if the measuring is properly done. Add one tablet to the first tube, two to the second, and so on. The tablets should be crushed in the measuring cup with the end of the spatula handle before adding to the soil in the test tubes, unless they are soft enough to dissolve quickly. Then add 25 cc. of water to each sample, shake until tablets are completely dissolved, let settle, add bromthymol blue, and read the endpoint as in the laboratory method

The tablets, as described in the foregoing, can be made satisfactorily by manufacturing pharmacists. A trial lot made by Parke, Davis and Company were practically constant in neutralizing value. Different batches might vary slightly and, therefore, require standardization.

In the field method, the addition of increasing amounts of Na_2CO_3 for titration is accompanied by increases in the amount of KCl used. This, it has been found, does not appreciably influence the results, as compared to the

laboratory method in which a constant quantity of KCl is used with varying amounts of base.

In case half-ton intervals are desired in the field method, tablets could be made to contain 0.25 gm. KCl and 0.0031 gm. $Na_2CO_3 \cdot H_2O$.

In the foregoing directions, no account has been taken of impurities in the limestone or other factors inimical to its effectiveness. As a matter of fact the tablets now in use at this station have been corrected for these factors, each tablet being equivalent to 1 ton an acre of average commercial limestone screenings as used in Illinois, the amount of $Na_2CO_3 \cdot H_2O$ present in each tablet being only 60 per cent of the theoretical amount. The reason for this reduction in amount has already been discussed.

APPLICATION OF THE METHOD TO A FIELD-TESTING PROGRAM

This quantitative field method is adapted for use in conjunction with the field testing and mapping plan as used in the Illinois soil extension program (7). This plan, which is being used successfully among Illinois farmers, requires the testing of 23 soil samples, uniformly distributed over a 40-acre field, by means of the potassium thiocyanate test. As a result of these tests, the field is mapped into areas of possibly high, medium, low, and no acidity. The interpretation of the three degrees of acidity, in terms of the amounts of limestone needed, is largely a matter of experience. Years of experience on the part of extension workers has resulted in a practical calibration of the test, and a color chart is now being used which has been found to be suitable for the average soil conditions. The deviation from this average condition, however, is likely to be large in certain soil types, and cannot be recognized even by the experienced soil tester.

The carrying out of the following plan enables one to make a definite quantitative interpretation of the thiocyanate test or any indicator test which may be used. All the samples from a given field on the same or related soil types showing the same degree of acidity by the qualitative test are composited. This gives a maximum of three samples from any field to be tested by the titration method, i.e., the "low acidity" samples, the "mediums," and the "highs." Thus the exact meaning of "low," "medium," and "high" acidity as mapped on this particular field is found in terms of pounds of limestone needed per acre. The areas testing non-acid by the thiocyanate test where proper precautions are taken[7] need no limestone and a further quantitative test would be superfluous.

The field method, as described in this paper, is not sufficiently rapid to be used on a large number of samples as is the thiocyanate test. Therefore, it is recommended that the thiocyanate test or similar indicator test be used for

[7] Certain soil types which are acid do not give a red color with the thiocyanate test unless a small amount of finely divided iron is added before shaking the mixture. This deficiency in that form of iron which gives the test seems to be associated with mature soils formed under conditions of good drainage.

the qualitative separation and mapping of the field so that the number of samples to be run by the titration method on a given 40 acres will be reduced to three or less. By taking advantage of both of these methods, a quantitative value is found for a rather large area with a minimum number of samples.

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THE LAWS OF SOIL COLLOIDAL BEHAVIOR: VI. AMPHOTERIC BEHAVIOR[1]

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The amphoteric nature of certain soil colloids has already been established by cataphoresis (2), by the Donnan distribution of free ions between the micellar and intermicellar solution (5), and by the adsorption of cations and anions at various pH values (3).

For an understanding of the soil forming processes, for an interpretation of the physical and chemical properties of different soils, and for a prediction as to the reaction of a soil to any particular treatment nothing can be more important than a knowledge of the various factors of which the amphoteric behavior of soil colloids is a function. The present section will therefore be devoted to a discussion of this behavior. For this purpose a series of colloids covering a wide variation in composition has been selected.

ADSORPTION OF ANIONS AND CATIONS AND CATAPHORESIS AT VARIOUS pH VALUES

The materials used include the colloidal fraction separated by the supercentrifuge from the following soils: (*a*) a Sharkey clay from Mississippi, (*b*) a Sassafras silt loam subsoil from Maryland, (*c*) a Cecil clay loam from Georgia, and (*d*) a Nipe clay from Oriente Cuba, and in addition a sample of bentonite from Rock River, Wyoming. The latter sample included the sand and silt.

Table 66 gives the composition of the soil colloids, two of which, the Sharkey and the Sassafras, have been reported in a previous publication (2). The sample of the Cecil colloid is identical with sample 4 in the tables of Robinson and Holmes (10). The Nipe colloid was separated from a sample of the Nipe clay which was obtained through the courtesy of W. O. Robinson. The composition of this colloid is assumed to be the same as that of another sample of colloid extracted from the same soil by the Bureau of Chemistry and Soils (analysis by Glen Edgington).

The soil colloids were all subjected to a prolonged electrodialysis until practically all of the diffusible ions were removed. Equal weights of the dry, finely ground colloid were placed in Erlenmeyer flasks to each of which was added 100 cc. of a solution of ammonium salt. The chloride, sulfate, and phosphate were studied. Series in which the pH values ranged from high to

[1] Journal Science paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

low were obtained by adding an excess of ammonia on the one side and an excess of the respective acid on the other. The NH_4 ion was employed because of the solubility of its salts with the different acids and because of its simple

TABLE 66

Composition of the Soil Colloids

NAME OF COLLOID	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	MnO	CaO	MgO	K_2O	Na_2O	P_2O_5	SO_3	IGNITION LOSS	ORGANIC MATTER	$\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
Sharkey....	52.05	0.51	22.52	8.12	0.04	1.36	2.52	1.89	0.21	0.64	0.20	9.99	3.92	3.18
Sassafras...	41.14	0.70	29.26	12.73	0.03	0.53	1.07	1.35	0.42	0.08	0.20	14.08	1.59	1.89
Cecil.....	33.95	0.62	36.06	11.02	0.15	0.31	0.40	0.56	0.44	0.25	0.06	16.67	2.25	1.34
Nipe.....	10.19	0.20	15.84	62.51	0.74	0.23	0.05	trace	trace	0.06	0.19	9.86	....	0.31

TABLE 67

Adsorption of NH_4, Cl, SO_4, and PO_4 ions by the Nipe Soil Colloid (3 gm. in 100 cc. solution)

M.EQ. ADDED		M.EQ. PER GRAM ADSORBED		CATAPHORESIS	pH
NH_4	Cl	NH_4	Cl	μ/sec. lv./cm.	
2.242	2.00	0.039	0.00	−1.30	7.25
2.121	2.00	0.021	0.003	−0.30	6.7
2.00	2.00	0.014	0.011	+0.47	6.1
2.00	2.10	0.012	0.024	+0.82	5.8
2.00	2.20	0.006	0.038	+1.51	5.3
2.00	2.40	0.007	0.059	+1.78	4.05
	SO_4		SO_4		
2.242	2.00	0.042	0.00	−1.44	7.2
2.121	2.00	0.032	0.007	−1.32	6.9
2.00	2.00	0.017	0.029	−1.08	6.65
2.00	2.10	0.017	0.046	−0.87	6.25
2.00	2.20	0.012	0.066	−0.61	5.9
2.00	2.40	0.011	0.105	±0.00	5.0
	PO_4		PO_4		
2.00	3.00	0.109	0.297	−2.02	7.5
2.00	4.20	0.093	0.408	−1.78	6.75
2.00	5.40	0.077	0.465	−1.68	6.1
2.00	6.60	0.055	0.561	−1.51	5.5
2.00	7.80	0.028	0.750	−1.00	4.6
2.00	9.00	0.017	0.921	−slight	3.8

determination. A minimum of 2 m.eq. per 100 cc. was chosen because at this concentration a fairly complete flocculation was brought about up to a pH of about 7.0, even in the case of the phosphate. The suspensions were

shaken in the machine at intervals for two days and then let stand until a complete or nearly complete sedimentation was brought about. The supernatant liquid was then analyzed in 25-cc. aliquots. The pH was determined

TABLE 68

Adsorption of NH_4, Cl, and PO_4 ions by the Cecil Soil Colloid (1 gm. in 100 cc. solution)

M.EQ. ADDED		M.EQ. PER GRAM ADSORBED		CATAPHORESIS	pH
NH_4	Cl	NH_4	Cl	μ/sec. lv./cm.	
2.605	2.00	0.207	0.00	−2.02	8.4
2.121	2.00	0.123	0.00	−1.21	5.9
2.00	2.00	0.074	0.004	−0.47	4.5
2.00	2.10	0.046	0.016	+0.28	4.0
2.00	2.20	0.044	0.020	+0.38	3.6
2.00	2.40	0.040	0.032	+0.38	3.1
	PO_4		PO_4		
2.605	3.00	0.237	0.186	−2.33	8.2
2.121	3.00	0.209	0.291	−1.85	7.4
2.00	3.00	0.202	0.300	−1.85	7.3
2.00	3.30	0.204	0.336	−1.78	6.9
2.00	3.60	0.204	0.324	−1.68	6.7
2.00	4.20	0.208	0.354	−1.68	6.5
2.00	5.40	0.152	0.423	−1.26	5.8
2.00	7.80	0.044	0.813	+ slight	3.1

TABLE 69

Adsorption of NH_4, Cl, and PO_4 ions by the Sassafras Soil Colloid (3 gm. in 100 cc. solution)

M.EQ. ADDED		M.EQ. PER GRAM ADSORBED		CATAPHORESIS	pH
NH_4	Cl	NH_4	Cl	μ/sec. lv./cm.	
2.968	2.00	0.287	0.00	−2.33	7.05
2.484	2.00	0.173	0.007	−0.76	5.4
2.00	2.00	0.065	0.020	+0.43	4.1
2.00	2.20	0.055	0.027	+0.50	3.8
2.00	2.40	0.048	0.027	+0.61	3.4
2.00	2.80	0.047	0.033	+0.76	3.0
	PO_4		PO_4		
2.484	3.00	0.350	0.267	−2.45	6.75
2.242	3.00	0.327	0.303	−2.33	6.55
2.00	3.00	0.307	0.321	−2.16	6.4
2.00	4.50	0.269	0.456	−2.03	5.7
2.00	6.00	0.207	0.678	−1.90	4.3
2.00	9.00	0.112	1.089	−0.89	3.2

colorimetrically and the cataphoresis measurements were made in the remaining suspension. The adsorption of cations and anions at various pH values and the cataphoresis of the particles are shown in tables 67 to 71.

CATION ADSORPTION

Let us first consider the adsorption by the Nipe soil colloid. This material is very high in sesquioxides, especially iron, and is low in silica. The organic matter is also low, as indicated by the relatively small loss on ignition, and the P_2O_5 content is low. The quantity of acidoid constituents of the complex

TABLE 70

Adsorption of NH_4, Cl, and PO_4 ions by the Sharkey Soil Colloid (2.8 gm. in 100 cc. solution)

M.EQ. ADDED		M.EQ. PER GRAM ADSORBED		CATAPHORESIS	pH
NH_4	Cl	NH_4	Cl	μ/sec. lv./cm.	
4.178	2.00	0.661	0.00	−2.16	6.8
3.089	2.00	0.376	0.00	−1.68	5.6
2.00	2.00	0.111	0.001	−1.01	3.2
2.00	2.20	0.090	0.001	−0.60	3.1
2.00	2.40	0.081	0.001	−0.43	3.0
2.00	2.80	0.066	0.004	−0.25	2.8
	PO_4		PO_4		
3.210	3.00	0.674	0.324	−2.25	6.5
2.605	3.00	0.592	0.363	−2.18	6.1
2.00	3.00	0.448	0.387	−2.05	4.8
2.00	4.50	0.332	0.474	−1.92	4.0
2.00	6.00	0.245	0.606	−1.68	3.3
2.00	9.00	0.164	0.810	−1.23	2.9

TABLE 71

Adsorption of NH_4, Cl, and PO_4 ions by Bentonite (3 gm. in 100 cc. solution)

M.EQ. ADDED		M.EQ. PER GRAM ADSORBED		CATAPHORESIS	pH
NH_4	Cl	NH_4	Cl	μ/sec. lv./cm.	
4.420	2.00	0.675	None	−2.33	8.2
2.00	2.00	0.133	None	−0.76	2.8
2.00	2.80	0.100	None	−0.48	2.1
	PO_4		PO_4		
3.452	3.00	0.633	0.221	−2.40	6.9
2.00	3.00	0.378	0.264	−2.12	4.2
2.00	9.00	0.172	0.360	−0.98	2.6

is therefore small whereas the basoids are present in great excess. This expresses itself in a relatively very low cation adsorption and in a high anion adsorption.

This colloid contains about one-fifth as much silica as the Sharkey colloid and yet it adsorbs only about one-sixteenth of the cations adsorbed by the

latter at a pH of 7.0. We have seen that the cation adsorption is not a linear function of the percentage of acidoid present. The greater the percentage of acidoid in the complex, the greater, relatively, its activity. We might distinguish between three conditions of the acidoid in the colloidal complex. One part is locked up within the complex and is therefore inactive. Another part exists in the surface or adsorption layer, but a fraction of this part is linked up with the basoid constituents leaving only the valences of the remaining fraction free to enter into exchange reaction with diffusible cations. In a complex like that of the Nipe colloid in which the basoids greatly predominate there is apparently only a relatively very small fraction of the acidoid in the active condition.

We note no significant difference in the adsorption of the NH_4 cation in the chloride as compared to the sulfate solutions but there is a decided increase in the cation adsorption in the phosphate solution. By a rough interpolation we find that although the colloid adsorbs less than 0.04 m.eq. NH_4 per gram at a pH of 7.0 from the chloride and sulfate solutions, it adsorbs about 0.1 m.eq. NH_4 per gram at the same pH from the phosphate solution. At a pH of 6.1 the adsorption has sunk to about 0.014 m.eq. in the chloride and sulfate solutions while it is still as high as 0.077 m.eq. in the phosphate solution.

It has previously been shown (8) that the phosphated sesquioxides possess a very high cation exchange capacity. It is therefore quite obvious that this increase in the NH_4 ion adsorption is due to the phosphate ions which are adsorbed in large quantities. The acidoid content of the complex has been increased through the adsorption of a polyvalent anion. The PO_4 ion displaces OH and, as we shall see later, also silicate ions in the complex. But not all of the three phosphate valences become engaged in this displacement. One or two of the valences remain "free," that is, exist in combination with diffusible and exchangeable cations. The electronegative acidoid valences in the complex have been increased and this has resulted in a higher cation exchange capacity and in a displacement of the isoelectric point to the acid side.

The reaction might be represented as follows:

$$(NH_4)_2HPO_4 + Al(OH)_3 \rightarrow \begin{matrix} OH \\ OH \end{matrix}\!\!\rangle Al - PO_4 \langle\!\!\begin{matrix} H \\ NH_4 \end{matrix} + NH_4OH$$

or

$$(NH_4)_2HPO_4 + OH - Al = SiO_3 \rightarrow H - PO_4 = Al - SiO_3 - NH_4 + NH_4OH$$

or by any other of several possible forms of the reaction. It must be remembered that a colloidal particle 0.1μ in diameter holds several millions of sesquioxide and silica molecules. The soil colloidal micelle represents a highly multivalent amphoteric ion and is capable of forming a practically infinite number of combinations merely with the common ions present in the soil solution.

ANION ADSORPTION

Turning now to the anion adsorption we note (*a*) that this increases as the pH decreases, (*b*) that the relative magnitudes in the adsorption of the three ions is $Cl < SO_4 < PO_4$, and (*c*) that the PO_4 ion is the only ion adsorbed from an alkaline solution. This is all in agreement with the author's previously published data (3).

This means that, of these anions, only the PO_4 ions are able to displace the OH ions in the complex against an OH ion contraction in the solution greater than that of the neutral point. The SO_4 and especially the Cl ions are so highly dissociated by the complex that they become totally displaced once the OH ions attain a certain small concentration. (In the case of the sesquioxides themselves, especially aluminum hydroxide, the SO_4 ion persists in the complex in considerable quantities up to a pH of about 8.0, i.e., the isoelectric point, as shown in section III of this series.)

CATAPHORESIS

If we now turn our attention to the speed of electrical migration of the particles we will find an interesting and illuminating expression of the amphoteric nature of soil colloids. We are still considering the Nipe colloid (table 67).

In the chloride solutions the colloid changes from negative to positive as the pH is decreased, the isoelectric point being somewhere between pH 6.1 and 6.7. In the sulfate solutions the colloid becomes isoelectric at pH 5.0. At still lower pH values the particles would be positive but because of the suppressing effect of the free divalent SO_4 anions the positive charge would remain low. In the phosphate solutions the particles remain negative at a pH as low as 3.8.

The reasons for the negative charge being higher and maintaining itself at a lower pH in the sulfate and especially in the phosphate systems are twofold. In the first place the free divalent SO_4 ions and the di- and trivalent HPO_4 and PO_4 ions suppress the negative charge (resulting from the cationic dissociation) less than the monovalent Cl ions. In the second place the dissociation of the SO_4 ions and (at the pH range in question) especially the phosphate ions is much smaller than that of the Cl ions. It requires, therefore, a greater adsorption of SO_4 and PO_4 ions and a lower pH for the anionic dissociation to balance the cationic dissociation and render the complex isoelectric. The phosphated complex has in addition become more electronegative through an increase in the cation content.

DISSOCIATION

Some information in regard to the relative degree of dissociation of the diffusible anions and cations in the complex may be gained from the figures in the tables. It will be seen that in the chloride solutions the change of charge from negative to positive takes place before the quantity of Cl ions adsorbed equals the quantity of NH_4 ions. If the isoelectric point is that point at which

the anionic and cationic dissociations are equal then it is obvious that the Cl ions are more highly dissociated by the complex than are the NH_4 ions. In the sulfate solution the colloid is isoelectric at a pH where 0.011 m.eq. NH_4 ions and 0.105 m.eq. SO_4 ions are adsorbed. Evidently the SO_4 ions must be much less dissociated by the complex than the NH_4 ions. In the phosphate solution the colloid is still negative at a point where the adsorbed anions are in still greater excess over the adsorbed cations. The phosphate ion is therefore dissociated least of all. (At low pH values, below 3.0, when the phosphate ion is monovalent its effect upon the charge is more like that of the Cl ion.)

AMPHOTERIC BEHAVIOR IN RELATION TO COMPOSITION

If we now make a general review of tables 67–71 it will be noted that the various colloids adsorb very different quantities of anions and cations and behave, accordingly, differently in the electric field.

With an increase in the silica content (or acidoid content in general) of the colloid we find an increase in the cation adsorption and a decrease in the anion adsorption. As the silica/sesquioxide ratio (or the acidoid/basoid ratio in general) increases the colloids assume a more pronounced electronegative character with their isoelectric point displaced more and more to the acid side.

The Sharkey colloid adsorbs only very small quantities of Cl ions even at low pH values and remains negative through the entire range of pH values down to 2.8. At this point the negative charge is quite low and it is possible that the material would be isoelectric at a still lower pH, but at such high acidities the decomposition of the complex becomes so great that any figures obtained will be of doubtful value.

Soil colloids high in silica, like the Sharkey colloid, react with more Cl ions in acid solutions than is apparent in the analysis. Such soil colloids are very unstable in the unsaturated condition, i.e., in acid solution, yielding considerable quantities of silica, aluminum, and iron. As the Cl ions displace the OH ions in the complex, soluble products are formed which obviously possess a variable composition but may be of the following forms:

$$Cl - Al = SiO_3,\ Cl - Al \begin{matrix} -\ OH \\ -\ SiO_3H, \end{matrix} \quad (OH)_2\,Al\,Cl,\ Al\,Cl_3,\ H_2SiO_3$$

etc. all depending upon the pH. A soil complex possessing more silica (or acidoid in general) than the Al and Fe can normally hold in combination can be stable only as long as it is saturated with divalent cations. This question will be dealt with later.

It is to be noted that the Sharkey colloid adsorbs considerable quantities of the PO_4 ions and that, at the two highest pH values (6.1 and 6.5), this colloid adsorbs more PO_4 than either the Sassafras or the Cecil colloids. This "irregularity" was observed several years ago (1925). The adsorption of the PO_4 ion from 0.02 molar solutions of phosphoric acid and of neutral potas-

sium phosphate by the Norfolk and Sharkey soil colloids, by humus, and by kaolin was studied. The results are shown in table 72.

The Sharkey colloid adsorbed more PO_4 from the neutral phosphate than did the Norfolk colloid. It had previously been found that the adsorption of the Cl, SO_4, and PO_4 ions from acid solution increased in general with a decrease in the silica sesquioxide ratio (3). The result of this experiment appeared anomalous and could not then be explained, and the work was put aside for future study. We see that the untreated Sharkey colloid containing much exchangeable Ca and Mg adsorbed no more PO_4 than did the electrodialyzed colloid. These cations could therefore not be responsible for the PO_4 ion adsorption [Gedroiz (1)] nor for the differences in the adsorbing power of the two colloids. Another explanation must be found.

Soil colloids like the Sassafras, the Norfolk, and the Cecil possess undoubtedly more displaceably OH ions than the Sharkey colloid, since the latter is poorer in basoid constituents. The former adsorb, therefore, more PO_4 ions

TABLE 72

Adsorption of PO_4 ion from 0.02 molar solutions of phosphoric acid and of neutral potassium phosphate by Norfolk and Sharkey soil colloids, by humus, and by kaolin

MATERIAL	$\frac{SiO_2}{R_2O_3}$	M.EQ. PO_4 PER GRAM ADSORBED FROM	
		H_3PO_4	Potassium phosphate
Electrodialyzed Norfolk colloid	1.59	.697	.286
Electrodialyzed Sharkey colloid	....	.634	.445
Untreated Sharkey colloid	3.18	....	.445
Humus	....	None	None
Kaolin	2.0(?)	None	None

from an acid solution because in acid solutions it is primarily the OH ions which suffer displacement. The high H-ion concentration suppresses the OH-ion concentration in the solution to such a low value that the phosphate ions, which were present in large numbers, compete successfully for a place in the complex. The displacement will be governed by the "solubility product" of each combination or, since the term "solubility product" can hardly be applied to a colloidal ionogen, we might rather state that the displacement will be governed by the ionic product of each combination.

It is very difficult to express reactions as complex as those of colloidal materials but the following scheme might serve to illustrate the displacement of OH by PO_4 ions in acid solution:

$$[Al_x(SiO_3)_y(OH)_z] \rightleftarrows n\,OH^- + [Al_x(SiO_3)_y(OH)_{z-n}]^+$$

$$H_3PO_4 \rightleftarrows 2H^+ + HPO_4^{--}$$

$$\downarrow\uparrow \qquad \uparrow\downarrow$$

$$n\,H_2O + [Al_x(SiO_3)_y(HPO_4)_{\frac{n}{2}}(OH)_{z-n}]$$

This reaction will be displaced to the right by an increase, and to the left by a decrease in the H-ion concentration. At high pH values the OH ions are only slightly displaced by other anions because the OH ions are firmly associated, i.e., slightly dissociated by the complex. But we have seen (section IV) that the PO_4 ions displace very readily the silicate ions in the synthetic complex. Now if this displacement takes place in the natural colloids as well, then the aforementioned anomaly might easily be explained. The displacement of the silicate by the phosphate ion would be less affected by the pH and would take place in alkaline as well as in acid solution. Highly silicated colloids, like the Sharkey, might therefore show a higher phosphate adsorption in neutral and alkaline solutions than soil colloids poor in silica and rich in sesquioxides.

In the foregoing experiments the displaced silica was not estimated but in other, specially made tests, it has been found that the phosphate ions displace considerable quantities of silica from the Sharkey colloid and from bentonite, whereas this displacement is much less in the other colloids which are poor in silica and rich in sesquioxide. These results will be reported later.

The displacement of the silicate ion, by the phosphate ion might be illustrated thus:

$$\begin{array}{rcl} [Al_x(OH)_z(SiO_3)_y] & \rightleftarrows & n\,SiO_3^{--} + [Al_x(OH)_z(SiO_3)_{y-n}]^{++} \\ Na_2HPO_4 & \rightleftarrows & 2Na^+ + HPO_4^{--} \\ & & \downarrow\uparrow \qquad \Updownarrow \\ & & n\,Na_2SiO_3 + [Al_x(OH)_z(HPO_4)_n(SiO_3)_{y-n}] \end{array}$$

The symbols within the brackets represent the non-diffusible complex. Every ion in the complex must be capable of some degree of dissociation. This is evident from the reactivity and instability of the complex. But it is only the common, easily displaceable mono and divalent ions which are capable of a high degree of dissociation and which, through this ability to dissociate, are responsible for the electrokinetic and other related behaviors of the colloid.

If we now turn to table 71 we will note that bentonite adsorbs no Cl ions and less PO_4 ions than any of the other materials. The bentonite, on the other hand, maintains, at very low pH values, a greater cation adsorbing capacity and a stronger negative charge than any of the soil colloids. That 0.172 m. eq. of NH_4 ions per gram bentonite can enter the complex at a pH of 2.6 indicates an acid of considerable strength. The relatively low phosphate adsorption by the bentonite may be ascribed to its low iron content.

It must finally be pointed out that no linear relationship should be expected between any property and a ratio like that of silica to sesquioxides. The variations in this ratio express only one of several variations which greatly influence the behavior of the colloid. The linkage between the constituent parts, e.g., the degree of hydrolysis, may be very different. Then there is the variation in the quantities of Al and Fe in the complex. These ions differ greatly in respect to the dissociation and electrokinetics of their compounds. The pres-

ence or absence of other acidoids such as humus and phosphoric acid will greatly affect the behavior. Crystallization and particle size are other factors. Thus kaolin adsorbs practically no anions or cations whereas soil colloids having the same silica/sesquioxide ratio adsorb both kind of ions in considerable quantities. Many anomalies will therefore be encountered but these are of value and should be studied as to their cause, for very often the most fundamental truths are thus established.

EXCHANGE ACIDITY, EXCHANGE NEUTRALITY, AND EXCHANGE ALKALINITY

The fact that soil colloids are amphoteric and that the cation adsorption increases with the pH while the anion adsorption increases as the pH decreases leads to a very interesting and significant condition not heretofore recognized. If a soil colloid is rendered unsaturated (i.e. free acid-base ampholytoid) by displacing all the diffusible anions and cations by OH and H ions as in electrodialysis, and then treated with a neutral salt solution, an exchange acidity is (usually) developed. This has long been known. But if, instead of a neutral salt solution, the pH of the solution be adjusted, by adding free acid, to that value at which the displacement of the OH ions in the complex by the anions of the salt just balances the displacement of the H ions in the complex by the cations of the salt, then the pH of the solution will not be affected by the colloid. This pH we will call the point of exchange neutrality. If we use a solution of a still lower pH we shall find that the pH is increased by the colloid. We have then an exchange alkalinity.

The importance of this behavior, peculiar to all amphoteric colloids, will be recognized without much emphasis. Thus the pH, at equilibrium, of a suspension of an amphoteric soil in a neutral salt solution can be no measure of the degree of "unsaturation" or of the "lime requirement." Every soil which reacts amphoterically with the ions of the salt will yield OH as well as H ions to the solution. The resulting pH will therefore represent the algebraic sum of the numbers of H^+ and OH^- ions displaced. Whether the pH will be higher or lower than the pH of the colloid in water will depend upon which of the two ions are displaced in the greater numbers. The pH of exchange neutrality will not only vary with the composition, i.e., the amphoteric character, of the colloid but also with the nature of the ions of the salt employed. The figures in tables 67–69 show this quite plainly.

If we assume that the adsorbed cations (NH_4) displaced nothing but H ions (which might not be strictly true although the colloid was electrodialyzed) and that the adsorbed anions displaced nothing but OH ions (which is not entirely the case, for it was found that the phosphate ions, at least, displace some silicate ions) then, that pH at which the anion adsorption equals the cation adsorption would be the pH of exchange neutrality. For the Nipe colloid (table 67) this point lies below a pH of 6.1 in the chloride system and

above a pH of 6.65 in the sulfate system whereas in the phosphate system the anions are adsorbed in large excess even at a pH as high as 7.5.

The point of exchange neutrality lies, therefore, at a higher pH the greater the energy of displacement of the anion and is therefore not to be confused with the isoelectric point which lies at a lower pH the greater the energy of displacement of the anion, i.e. the less the anion is dissociated by the complex. We would naturally conclude that the energy of displacement of anion will be in inverse proportion to the dissociation of the compound which the ion forms with the complex. That this actually is the case is born out by the

TABLE 73

Exchange reactions of soil colloids in N NaCl solutions at various pH values

H adjusted by HCl—0.5 gm. soil colloid in 10 cc. and 0.25 gm. humus in 30 cc. solution

ORIGINAL NaCl SOLUTION pH	pH AFTER EQUILIBRIUM WITH COLLOIDS											
	Nipe		Cecil		Sassafras		Sharkey		Bentonite		Humus	
	pH	Difference	pH	Difference	pH	Difference	pH	Difference	pH	Difference	pH	Difference
7.9	6.8	−1.1	4.45	−3.45	4.2	−3.7	2.95	−4.95	2.3	−5.6	3.1	−4.8
6.85	6.6	−0.25	4.4	−2.45	4.2	−2.65	2.95	−3.9	2.3	−4.55	3.1	−3.75
5.55	6.05	+0.5	4.4	−1.15	4.2	−1.35	2.9	−2.65	2.3	−3.25	3.1	−2.45
4.4	6.0	+1.6	4.35	−0.05	4.2	−0.2	2.9	−1.35	2.3	−2.1	3.05	−1.35
3.6	5.9	+2.3	4.3	+0.7	4.15	+0.55	2.9	−0.7	2.2	−1.4	2.9	−0.7
2.2	4.25	+2.05	3.7	+1.5	3.5	+1.3	2.8	+0.6	1.8	−0.4	2.25	+0.05
1.5	....	.....	....	.....	....	.....	....	.....	1.2	−0.3	1.9	+0.4
pH of 1:10 water extract.....	6.0	.....	4.8	.....	4.7	.....	3.6	.....	2.7	.....	.....	.....
pH of 1:10 suspension.	6.65	.....	4.75	.....	4.63	.....	3.73	.....	2.60	.....	3.72	.....

figures in the tables. The energy of displacement, i.e., the order of quantity in which the various anions enter the complex at a given pH is:

$$PO_4 > SO_4 > Cl$$

On the assumption that the isoelectric point is that point at which the anionic and cationic dissociation are equal, we obtain the following order of dissociation

$$Cl > SO_4 > PO_4$$

The result of this is that anions which are slightly dissociated by the complex and have therefore a strong displacing power, will displace the point of exchange neutrality to a higher pH whereas the point of electro-neutrality is, for the same reasons, displaced to a lower pH. The reverse will be true of the cations when paired to a common anion.

General principles like these must, because of their universality, prove most useful and serve to place soil chemistry on a more firm scientific basis.

In table 73 are shown the exchange reactions of the various soil colloids, including humus, with normal NaCl solutions. A series of NaCl solutions at various pH values were prepared by adding NaOH or HCl. Half a gram of the air-dry materials was added to 10 cc. of each of the solutions, except in the case of the humus where 5 cc. of a stock suspension of electrodialyzed humus containing 0.25 gm. were placed in 25-cc. salt solutions. The suspensions were shaken for about half an hour and let stand over night. The pH of the supernatant liquid was then determined as noted.

In reviewing the results the following points should be noted: (*a*) the pH of exchange neutrality is not identical with the isoelectric pH although in the case of two highly, and perhaps more nearly equally, dissociated ions like the Na and Cl ions the two points might be very close together. (*b*) Although the pH values of exchange neutrality in the case of the Nipe, Cecil, and Sassafras colloids coincide fairly well with the isoelectric pH values in the NH_4Cl systems in tables 67–69, no comparison as to the proximity of the two points is permissible. We are dealing with the same colloids but not with the same complexes and this distinction cannot be overstressed. In the one case we have an ammonium and chloride complex and in the other case we have a sodium and chloride complex and the properties of each may be expected to vary as much as the properties of two different phosphates. (*c*) It is assumed that all ordinary electrolytes were removed by electrodialysis and that the salt reacted only with the free ampholytoid. It is fully recognized that where, for example, the added NaCl + HCl mixture reacts with compounds like phosphates and silicates we will get an increase in the pH, an apparent exchange alkalinity, because of the formation, by displacement, of a less dissociated acid independent of any displacement of OH ions.

It would perhaps have been better to add various amounts of acid to the colloid suspensions, determine the pH, then add the neutral salt solution (as was done in the experiment reported in section V) or the neutral salt in the dry form, and again determine the pH.

Nevertheless there can be no doubt about the significance of the figures in table 73, supported as they are by the quantitative adsorption data of tables 67–71. The differences in the points of exchange neutrality of the different colloids are an expression of differences in their amphoteric nature. For a given pair of ions the pH of exchange neutrality will be higher the greater the quantity (or activity) of the basoid in the colloid and will be lower the greater the quantity (or activity) of the acidoid in the colloid.

The Sharkey colloid, which did not become isoelectric in the NH_4Cl system at a pH as low as 2.8, yields an exchange alkalinity at this pH, whereas the bentonite develops an acidity down to a pH of 1.2. These pH values are below those prevailing in any normal soil, even the most acid. The reactions are therefore not significant. Colloids like those of the Sharkey and of bentonite

evidently do not react amphoterically with chlorides and probably not, to any appreciable extent, with nitrates and sulfates either, under soil conditions. The amphoteric reaction with NaCl, apparent at low pH values in the case of the Sharkey is probably largely a reaction by the products of decomposition. It is obvious that any colloid consisting of iron or aluminum silicate will react amphoterically when decomposed since it consists of a weak acid and a weak base. With the phosphates all the mineral soil colloids react amphoterically over the entire range of normal soil reactions.

The humic acids have always been found to be electronegative down to the lowest pH values at which they have been examined. But it will be seen that the electrodialyzed humus develops an exchange alkalinity at a pH of 2.25. Whether this is due to some remaining humates (the ash content was 1.12 per cent) or to proteins has not been determined but this question will be taken up later.

THE ULTIMATE pH OF SOILS

Perhaps the most significant thing about the amphoteric nature of soil colloids and weathered minerals in general is its relation to, what might be called, the ultimate pH of the soil. The pH of a soil may, of course, vary widely and show no relationship whatsoever to the composition of the colloid. The pH may be very high as a result of the accumulation of Na_2CO_3 or it may be very low due to the presence of humic acids or to the oxidation of sulfur or pyrites or to the excessive application of $(NH_4)_2SO_4$, etc.

But with the exception of these abnormal conditions the pH must bear a definite relationship to the chemical nature of the soil complex. This relationship must express itself most clearly in the case of the electrodialyzed soil colloids. The electrodialyzed soil represents a thoroughly leached soil. In both processes the diffusible anions and cations are removed, except the OH and H ions which are always present in the water and can therefore not be removed. On the contrary the OH and H ions displace all other diffusible anions and cations in the complex as the electric current or the leaching water removes the latter ions to the vanishing point from the soil solution. The reaction might be represented thus:

$$\begin{array}{rcl} A\ [\text{complex}]\ \ B & \rightarrow & [^{+}\text{complex}]^{-} + A\ B \\ H_2O & \rightarrow & OH^{-} + H^{+} \\ & & \downarrow \quad \downarrow \\ & & OH\ [\text{complex}]\ H \end{array}$$

in which *A* and *B* stands for any diffusible anion or cation originally present in the complex.

The ultimate product of this reaction will be the free acid-base-ampholytoid and the ultimate pH will be determined by the relative strength of the acid and basic groups in the amphoteric complex. This again will depend upon the composition, that is, upon the ratio of acidoids to basoids. But it must be

pointed out that this ratio becomes in this connection an activity ratio rather than a mass ratio. It makes a difference whether the acidoid consists of silicates, phosphate, or humate groups and whether the basoid consists of Al or Fe. It has already been shown (sections III, IV, and V of this series) that these materials vary greatly in acidic and basic properties. The soil colloids here considered consist primarily of silica and sesquioxides. We might therefore expect a more or less definite relationship between the ultimate pH and the silica sesquioxide ratio. The variations in the ratio of Al to Fe will no doubt cause minor deviations as will also the variable but appreciable percentage of organic matter.

The pH values of the electrodialyzed colloids leave no doubt that the amphoteric nature of soil colloids determines their ultimate pH. The pH values of the 1:10 suspensions were determined by the quinhydrone electrode, and the pH of the water extracts were determined in the somewhat turbid supernatant liquid by the indicator method. Both series of values are shown in the last two rows in table 73. The quinhydrone pH of 6.65 of the Nipe colloid may be erroneous. This colloid contains considerable manganese in the presence of which the method is unreliable.

In the case of the other three colloids and of bentonite the pH is slightly higher in the (slightly turbid) water extracts than in the suspensions. That is, these colloids show a slight "suspension acidity" (Wiegner and Palmann). That the suspension effect is small may be accounted for by the proximity of the colloids to their isoelectric point. The Donnan equilibrium demands that the suspension effect must be acid in negative and alkaline in positive colloids as shown in the preceding section (8).

The soil colloids here studied are more strongly acidic than basic. If we express the acid dissociation constant of the acidoid group by K_{acidoid} and the basic dissociation constant of the basoid group by K_{basoid} in analogy to K_a and K_b of molecularly dispersed acids and bases then we may state that for soil colloids

$$K_{\text{acidoid}} > K_{\text{basoid}}$$

The free soil ampholytoid is therefore always acid in relation, but as the proportion of basoid increases in quantity and activity K_{basoid} becomes more nearly equal to K_{acidoid} and the reaction of the free ampholytoid will approach the neutral point as exemplified by the Nipe colloid.

Where

$$K_{\text{acidoid}} = K_{\text{basoid}}$$

the reaction of the free ampholytoid will be neutral.

Where

$$K_{\text{acidoid}} < K_{\text{basoid}}$$

as in free aluminum hydroxide we should find an alkaline reaction.

Since all the electrodialyzed soil colloids are acid in reaction they should also show a negative charge. This follows on the assumption that all diffusible anions and cations except OH and H ions were removed from the colloid. (This would be strictly true only in the case of a perfect ampholytoid, see the following.) If the OH and H ions are the only diffusible ions and if the H ions are more dissociated by the complex than the OH ions, that is,

$$K_{\text{acidoid}} > K_{\text{basoid}}$$

then the colloid must be negative in so far as the cataphoretic charge is governed by the dissociation.

This was found to be so even in the case of the Nipe colloid but in the latter instance only upon boiling off the CO_2 from the water. The ultimate pH of soil colloids, that is, the pH of the free ampholytoid, is therefore not the pH of the isoelectric point. The following relationship must exist: Free ampholytoid colloids in which $K_{\text{acidoid}} > K_{\text{basoid}}$, must be negative and their pH will be below neutrality, but above the isoelectric pH. If $K_{\text{acidoid}} < K_{\text{basoid}}$ then the free ampholytoids will be positive and their pH will be above neutrality but below the isoelectric pH. If $K_{\text{acidoid}} = K_{\text{basoid}}$ then the colloid will be neutral and isoelectric. Only in the last case, therefore, will the ultimate pH be identical with the isoelectric point.

THE ISOELECTRIC POINT

To render the free soil ampholytoid isoelectric, acid must be added. The effect of this will be as follows: The acid will combine with the basoid group forming a saloid (colloidol salt) plus water. A part of the acid will remain free and cause a lowering of the pH. This will suppress the dissociation (or activity) of the acidoid group. A point will ultimately be reached at which the anionic dissociation is equal to the cationic dissociation and the complex is isoelectric. If we use an acid like HCl, the anion of which is highly dissociated by the complex, comparatively small quantities will render the colloid isoelectric, whereas considerable quantities of H_2SO_4 and still more H_3PO_4 will be required to accomplish the same end because of the low dissociation of the complex in combination with these ions. This is all borne out by the preceding experiments.

The isoelectric point of ampholytes and amphoteric colloids (ampholytoids) is generally spoken of as if it were a fixed point. For ampholytes such as the amino acids which are soluble and which form highly dissociated salts with acids and bases this may be approximately true. But in the case of insoluble materials like the colloids this view is certainly erroneous. Because of the very high ionic density on the surface of a colloidal particle consisting of reactive material like the soil colloids, dissociation is very limited. (A colloidal particle 0.1μ in diameter may contain over half a million displaceable ions.) We have previously indicated that if all the diffusible and exchangeable cations on a soil colloidal particle were dissociated a potential far in excess of the

maximum potential between ions in a dilute solution would result. The colloidal complex can evidently not dissociate ions against a certain critical maximum in potential. It appears that the valence effect is very great in this relationship so that the critical potential is much lower for divalent than for monovalent ions and still lower for tri- and tetravalent ions.

Then there is the influence of the solubility of the compounds which the various ions form with the colloid. In the case of a soluble ampholyte we ought to find the same isoelectric pH whether we use HCl, H_2SO_4, or H_3PO_4; NaOH; or $Ca(OH_2)$, as the case may require, in adjusting the pH. The salts formed would all be highly ionized and if the solubility product of any of the salts formed should be exceeded, an insoluble part will be precipitated out and separated from all influence upon the dissolved fraction.

In colloids it is different. Here there is no dissolved phase. The colloid includes both the ionized and insoluble phase in one. We will therefore find a different isoelectric pH depending upon the solubility of the compound formed with the various ions we may add.[2] Thus if we displace the OH ions in the free soil ampholytoid by Cl ions, we do not have to lower the pH very much before the complex is isoelectric. The highly dissociated Cl ions soon balance the dissociation of the H ions. If, on the other hand we displace the OH ions by phosphate ions we do not alter the anionic dissociation appreciably, because of the low degree of solubility of the compound formed. The electrokinetic effect of the phosphate ions will, within a certain pH range, be opposite to that of the Cl ions. We find, at the same pH values, an increase instead of a decrease in the negative charge (see table 67 and also table 25, part II). The explanation is that we have displaced the OH ions by a polyvalent anion which itself forms an amphoteric colloid with Al and Fe. All of the PO_4 valences are not engaged by the basoid group. The "free" valences remain linked to the diffusible cations of the added phosphate. Instead, therefore, of increasing the anionic dissociation of the complex we increase the cationic dissociation. By the displacement of the OH ions we have suppressed a part of the basoid group while we have added to the acidoid group.

Thus the free ampholytoid

$$OH\ [complex]^-\ H^+$$

will be negative because the H-ion dissociation exceeds the OH-ion dissociation. With only a few of the OH ions displaced by Cl ions the complex

$$Cl^-\ [^+complex]^-\ H^+$$

will be isoelectric. If the OH ions are displaced by PO_4 ions then the complex will, at the same pH, be still more negative thus:

[2] Any combination of an ion with the colloidal complex cannot be said to be soluble. If it causes solution the dissolved part ceases to be colloid. But the union of Cl ions with the Al ions of the complex is potentially a soluble combination. It remains insoluble and colloidal only insofar as some of the Al valences remain linked up in an insoluble combination.

$$Na^+ [^-PO_4 = \text{complex}]^- H^+$$

At higher pH values the PO_4 ion exists as part of the nondiffusible colloidal complex whereas at lower pH values (below 3.0), when the phosphate ion is monovalent, it is dissociated by the complex, which then becomes isoelectric thus:

$$H_2PO_4^- [^+\text{complete}]^- H^+$$

From this it is obvious that no fixed point can be assigned as the isoelectric point of the amphoteric soil complex. This point will vary with the nature and concentration of the ions in the solution with which the complex exists in an exchange equilibrium. But we must not be led to assume that it is one and the same complex which is isoelectric at different pH values. The composition of the complex varies continuously with the composition of the solution.

The hydrogen-ion concentration (H^+) at the isoelectric point of soluble ampholytes is defined by Michaelis (9) thus

$$(H^+) = \sqrt{\frac{K_a}{K_b}} K_w$$

Where K_a and K_b represent the acid and basic dissociation constants of the ampholyte and K_w the dissociation constant of water.

Now it is obvious that this expression of the isoelectric point can only apply to soluble ampholytes which form completely dissociated salts but which dissociate to a limited extent as acids and bases. The H-ion concentration at that point at which the anions and cations of the ampholyte are equal i.e., the isoelectric point, will then depend upon the factors in the foregoing equation, which involves only the H and OH ions.

But if a compound which is only slightly dissociated is formed with ions other than the H and OH ions, then the iso-electric point ceases to be a function merely of the H-ion concentration. We have been presented with abundant evidence that the soil colloids form slightly dissociated compounds with a number of the common ions and we have attempted to explain the electrokinetic behavior on the basis of differences in dissociation of the compounds which the complex forms with the various ions. This explanation will, it is believed, account for the fact that, as Svedberg (11) points out, "measurements of cataphoresis show that the isoelectric point also depends on the other (other than H and OH) ions present."

The view is here held that the cataphoretic isoelectric point is the isoelectric point defined by Michaelis and represents that point at which the anionic and cationic dissociation of the ampholytoid is equal. But in a colloidal ionogen this point is governed to a greater or lesser degree by all the ions present and not alone by the H and OH ions.

DISSOCIATION CONSTANT

The dissociation of colloidal ionogens cannot be studied by the same methods as are applied to solutions. We cannot determine the concentration of the active mass and we can therefore not apply the mass-law. Some writers on soil chemistry believe that they have determined the acid dissociation constant of soil colloids and present the figures. But the relationship of the hydrogen-ion concentration in a suspension of a soil colloid to the dissociation constant is not so simple. The pH of a suspension is the resultant of the pH of two separate systems which are in a state of equilibrium with each other. One of these systems is a true solution, a dispersion of single, diffusible ions and molecules. The other is a dispersion of colloidal micellae. Each micelle consists of a colloidal particle which by dissociation becomes a highly multivalent ion complex and is surrounded by an atmosphere of diffusible ions which by electrostatic attraction are prevented from diffusing very far into the external medium. The concentration of these diffusible ions is apparently greater nearer the surface than further out in the micellar atmosphere (5, p. 206).

We have at present no direct method whereby we may determine the average micellar ion concentration. The most reliable information seems to be by way of the Donnan equilibrium. From the distribution of the free electrolyte between the gel and the outside solution the author calculated the degree of dissociation of the exchangeable cations in samples of soil colloids (5). It was found that the dissociation increased rapidly with the increase in the ionic strength of the solution. This indicates that the activity of the multivalent colloidal ion is greatly suppressed by the interionic attraction (6, p. 391).

To determine the dissociation constant of the colloidal acid or base and thus define their strength we must not only know the degree of dissociation but we must also know the valence of the colloidal ion and the concentration of the undissociated molecular species. In colloids this cannot be defined in ordinary units.

A PERFECT COLLOID

It is obvious that the concentration of any ion in a solution extracted from any colloid can give no information about the dissociation of the colloid. The ions in the solution represent the soluble part of the colloid or else they are products of hydrolysis. They may, of course, also belong to the solvent or to some extraneous electrolyte. These ions have no more to do with the colloidal state inasmuch as they are all paired with other diffusible ions and can, therefore, be separated from the colloid by filtration. The diffusible ions dissociated by the colloid are subjected to an electrostatic attraction, are not free to diffuse away from the particles, and can therefore not be separated from the colloid by mechanical means.

Probably all colloids, with the possible exception of carbon, are soluble to some extent. The solubility of soil colloids appears to decrease as they

approach the isoelectric point. The strongly electronegative colloids possessing a high silica content seem to be the most soluble. (This relationship is now being investigated.) Soil colloids, even after being subjected to a prolonged electrodialysis, continue, because of their solubility, to affect the reaction of their water extracts.

A perfect colloid would be entirely insoluble and if completely electrodialyzed would yield a neutral extract.

DIFFERENT KINDS OF AMPHOLYTOIDS

We might distinguish between three kinds of amphoteric colloids thus:

1. Those in which the acid and basic radicals are represented by a single atonic group as in $Al(OH)_3$.
2. Those in which the two radicles are spatically separated but attached to the same molecule as in the proteins.
3. Those which may be defined as amphoteric salts. The latter are insoluble compounds of di- or polyvalent weak acids with di- or polyvalent, weak bases. Because of the low dissociation of these acids and bases their salts are partly hydrolyzed.

These compounds contain residual H and OH ions and react therefore amphoterically: as bases at a low pH and as acids at high pH values. These compounds are insoluble acid and basic salts and may be represented by the following general formula:

$$H\,[A\ B]\,OH$$

All of these types of ampholytoids are present in most soils but the last type plays a dominating role in all but highly organic soils. This latter type is represented primarily by the silicates but also by the humates and phosphates of aluminum and iron. These compounds react not only with acids and bases to form water and the corresponding saloid but they react amphoterically also with salts as we have seen.

Whereas amphoteric colloids of the type of $Al(OH)_3$, $Fe(OH)_3$, $Zn(OH)_2$, etc., represent definite compounds in the free condition and possess a definite strength as acids and bases there is no such definiteness in the case of the amphoteric colloidal salts. Their composition may be anything from the free basoid to the free acidoid provided the latter remain insoluble in the free condition. Their strength as acids and bases will depend upon the dissociation constants of the residual (free) acid and basic fractions. The quantities of the residual acid and basic fractions will depend upon the relative proportion and the strength of the original acid and base. The greater the excess of one of the components the greater will be its residual fraction. But the greater the strength of the original acid and base the further will the neutralization proceed and the smaller will be the residual fractions.

We cannot have a colloidal ampholyte in which both the acid and basic properties are strong. If one is strong the other must be weak or else both will be intermediate. The total strength of the acid and basic groups is limited to the dissociation constant of water.

$$(H^+)\ (OH^-) = K_w = 1 \times 10^{-14}$$

The following relationship may be given:

(*A*) Insoluble salts of strong acids and bases will not contain any residual H or OH ions and will not react amphoterically. Their ultimate pH will be that of neutrality. Because of a difference in the solution tension of their two ions they may be positive or negative. Their isoelectric condition will therefore depend primarily upon the relative concentration of their common ions in the solution rather than upon the pH. Example: $BaSO_4$ (4).

(*B*) Colloidal salts of weak acids and bases:

1. The acid is stronger than the base (as has been the case in all the colloidal complexes studied, e.g., "silicates," "phosphates," and "humates"): The ultimate pH and the isoelectric pH will both be on the acid side. The greater the proportion of acidoid to basoid the lower will be the ultimate and the isoelectric pH values and the greater the cation exchange capacity. For a given molecular ratio of acidoid to basoid the ultimate and isoelectric pH values will be lower and the cation exchange capacity will be greater the weaker the relative strength of the basoid or the stronger that of the acidoid. This is all in perfect agreement with the behavior of the aluminum and ferric complexes studied in the preceding section (8). $Fe(OH)_3$ is a weaker base than $Al(OH)_3$ and the ferric complexes have lower isoelectric pH values and possess greater cation exchange capacities than the corresponding aluminum complexes.

2. The base is stronger than the acid: There should then result a reciprocal behavior, but thus far we have no experimental verification.

(*C*) Colloidal salts (or mixtures) of exceedingly weak acids and bases: The acidoid and basoid residue will be very great, that is, there will be complete hydrolysis. The mixture will react amphoterically in strong acid and basic solutions only. There will be no charge due to dissociation and the mixture ought to be isoelectric over a wide pH range. But it will undoubtedly behave like all other inert substances in water, including air bubbles, and carry a negative charge. The ultimate pH will be close to 7.0.

To avoid confusion it is well to give a more specific definition of the terms "acidoid," "basoid," and "ampholytoid." They refer to the colloidal, insoluble fraction of electrolytes which dissociate into H and OH ions and non-diffusible ion complexes as distinguished from acids, bases, and ampholytes which are molecularly dispersed and dissociate into diffusible anions and cations. The distinction is useful because to the latter we can apply the mass-law while this is not possible in the case of colloids.

THE ACTIVITY CONCEPT

The ratio of silica to susquioxides

$$\frac{SiO_2}{R_2O_3}$$

has often been related to various properties of the colloids. But this relationship must be greatly affected by the presence in the colloid of other acid constituents such as humic and phosphoric acids. It would therefore be more correct to write this ratio in the form

$$\frac{\text{acidoid}}{\text{basoid}}$$

since this would express the ratio of all the acid to all the basic constituents present. But even this ratio, which is a mass ratio, would not express the most significant value, namely, the relative strength of the acidic and basic constituents. Thus the various acidoids, such as silicic, humic, and phosphoric, present in the amphoteric soil complex all possess different activities. The same is true of the basic constituents. Aluminum and iron impart, as we have seen (7), different properties to the complexes in which they enter as basoid constituents.

Then there is the physical condition of the material to be considered. Two colloids of the same composition, with respect to their major constituents, do not necessarily show the same behavior. Changes in structure and surface as a result of ageing and crystallization greatly affect the properties of colloids. Extreme dehydration resulting in the formation of anhydrides will altogether destroy certain properties. Drying alone will bring about irreversible changes in the humic acids.

All this is not expressed in any molecular mass ratio based upon the ultimate composition of the colloid. Since most of the properties characteristic of the colloidal condition of matter are directly related to the activity of the constituent parts and since the activity of colloids is subject to a far greater variation than the activities of ions and molecules in a true solution, it will be necessary to take the activity into account whenever we try to correlate the composition to any property of the colloid.

The aforementioned ratio should properly be expressed as follows:

$$\frac{\gamma A}{\gamma B}$$

in which A and B represent the quantities of acidoid and basoid respectively and γ their activity coefficients.

How are we going to find a numerical expression for the activities of A and B? We cannot determine the dissociation constants k_{acidoid}, and k_{basoid}, nor even the ratio between them as may be done in the case of soluble ampholytes from the expression

$$[\text{H}] = \sqrt{\frac{K_a}{K_b}}\, K_w,$$

for we have seen that the isoelectric pH of a soil colloid is not a fixed point but varies with the nature of the ions in the solution. In fact we have found nothing constant about the soil colloids. Like living organisms, the only thing invariable about the colloids is their variation. The study of colloids becomes, therefore, a study of their variations and since we have always found their behavior to vary in an orderly relation to certain factors, we may be hopeful of learning more about them.

The writer would suggest the ultimate pH of a suspension as an index of the

acidoid strength of the colloid. As an index of the relative strength between acidoid and basoid the isoelectric pH of the electrodialyzed colloid in HCl or the pH of exchange neutrality in NaCl may be suggested.

SUMMARY

Soil colloids in which the ratio of silica (or other acid groups) to sesquioxides is low show a pronounced amphoteric behavior. When this ratio is high the materials do not react amphoterically with the neutral salt anions and cations within the usual pH range of soils. All soil colloids react amphoterically with the phosphates.

The isoelectric point is not a fixed point but varies with the dissociation of the compounds which the colloidal complex forms with the adsorbed ions.

The fact that the soil colloids react as acids above a certain pH and as bases below this pH leads to the development of an exchange acidity in the first case and an exchange alkalinity in the second case. The transition point between the two forms of reaction is termed "the point of exchange neutrality." Here the anion and cation adsorption balance each other. The point of exchange neutrality is not a fixed point but is governed by the energy of displacement of the anions and cations which displace the OH and H ions in the electrodialized free ampholytoid. Its relation to the isoelectric point is discussed.

Related to, and determined by, the amphoteric nature of soil colloids is their ultimate pH, defined as the pH of the completely electrodialyzed materials, i.e., the free acid-base-ampholytoids. The relation of the ultimate pH to the neutral point, to the isoelectric point, and to the acid and basic strength of the amphoteric colloids is discussed. Various types of amphoteric colloids are discussed.

The activity concept is applied to the

$$\frac{\text{acidoid}}{\text{basoid}}$$

ratio of soil colloids.

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SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: IV. INFLUENCE OF PROXIMITY TO ROOTS ON ABUNDANCE AND ACTIVITY OF MICROÖRGANISMS[1]

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HISTORICAL

In recent publications (64, 65, 66) it has been shown that plant development exerts pronounced effects upon the microbial population of the soil. The bacteria developing upon albumin agar and the bacteria closely related to *Bacillus radiobacter* were much more numerous in soil about plant roots than in soil free from roots. The activity of the organisms as measured by the rate of formation of carbon dioxide and nitrate was much greater in the soil about roots. Specific differences were noted with different plants, particularly between annuals and biennials, and these differences were correlated with certain characteristics of growth of the plants. Actinomyces and fungi responded less to the plants than did the bacteria. Considered as a whole, however, the increase in these organisms was statistically significant (67). The results obtained during the second year of growth of three of the biennials are presented elsewhere (67). These results indicated that there is no marked decrease in abundance and activity of microörganisms while the plants are in a vigorous vegetative condition. In fact, during the second year, greater numbers of bacteria were observed than at any period of the first year. Evolution of carbon dioxide remained at a high level and nitrification was more active than at any preceding period of observation.

The results obtained by Velich (76), Hoffman (22), Joshi (27), Wilson and Lyon (81), Löhnis (38), and Smith (63) have been discussed in a previous report (64) and it need only be mentioned here that their results indicate that bacteria are more abundant about roots of developing plants.

Negative results were obtained by Creuzburg (9) who could demonstrate no increase in microörganisms or biological activity about plant roots. Headden (15) concluded that plants even depress microbial activities in soils. This conclusion is merely speculative, in view of the fact that it is based not upon determinations of the abundance of organisms but upon determinations of

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amounts of carbon dioxide in soil air. The observations of Vande Velde and Verbelen (75) indicate that, in soil which was not treated previous to planting, the number of bacteria diminished during germination of seeds. The small number of observations made and the limitations of the method used render these results inconclusive.

The results recently reported by Gräf (14) are particularly pertinent in connection with those to be discussed in the following pages. She found much larger numbers of the general bacterial population and also of members of the Radiobacter group in soil about plant roots. Even more interesting is the fact that upon the root surfaces there appeared many times as many organisms as in the soil adjacent to the roots. This study included observations with eight different kinds of plants. She made attempts to determine the capacity of organisms in the soils and root materials to form ammonia from peptone, to fix nitrogen, to oxidize ammoniacal nitrogen, and to assimilate nitrate nitrogen. These results give no indication of the activity of organisms about roots, because the methods used were not suitable for deriving this information. The fact that no pronounced increase was noted in the activity of organisms about roots does not justify the idea that microörganisms are no more active on root surfaces than in soil free from roots.

There is considerable evidence to indicate that carbon dioxide is produced in greater amounts in soils supporting plant growth than in soils devoid of plants (30, 69, 32, 1, 33, 56, 2, 74; see also recent review by Thomas, 70). Furthermore, greater amounts are produced during advanced periods of vegetative growth, during blooming, or fruiting, and there is a pronounced decrease during degeneration of the vegetative portion of the plant. Metzger (44) observed similar changes in the concentration of bicarbonates about roots. Russell and Appleyard (58, 59) obtained less conclusive results but nevertheless believed that the growing crop is a factor affecting formation of carbon dioxide in soil.

Little information is yet available to indicate what portion of the increase in carbon dioxide about roots is the result of microbial activity. It has been shown previously (64, 65, 66, 67, 14) and will be indicated in the following pages that microörganisms are far more numerous about roots and on root surfaces than in soil devoid of roots. Fred and Haas (12) demonstrated that the activity of microörganisms on root surfaces greatly increases the solvent action exerted by roots.

In view of these facts and that the author noted that greater amounts of carbon dioxide were produced by soils obtained from the region of root development, it was concluded that " . . . . at least part of the increase in carbon dioxide in the soil air under plants and increase in bicarbonates about plant roots should be ascribed to microbial action" (66, p. 438). This statement and those accompanying it seem conservative, in view of the fact that no other explanation seems likely to account for the vital activities of the tremendous numbers of microörganisms found about roots. Thomas (70) considers that the total contribution to the carbon dioxide by microörganisms is insignificant compared

with the amount evolved during the growing season by the respiratory activity of the roots. The author still persists that, notwithstanding the evidence offered by Thomas to the contrary, the information which is available at the present time does not permit the statement in any definite terms of what portion of the carbon dioxide produced about roots is the product of microbial activity and what originates from respiration of the root cells. The evidence presented by Thomas is that obtained from Barakov (1), Headden (15), and Turpin (74), all of whom made determination of the amounts of carbon dioxide in the soil air. These measurements of necessity included the sum of the amounts of carbon dioxide originating from all the various sources within the soil and permit no distinction between those amounts arising through microbial action and those coming from respiration of root cells.

There are many factors affecting the concentration of carbon dioxide about plant roots and thus rendering difficult an accurate interpretation of results. The amount of carbon dioxide in soil is no adequate indication of the speed with which it is being formed. Factors modifying rates of diffusion will create differences as great as factors modifying production (41). It can be imagined that even under plant cover, in certain instances there may be less carbon dioxide than in fallow soil, as where the plant increases the rate of diffusion by such changes as lowering the water content and making the soil more porous by root penetration.

Measurements of gas formation in solution cultures of plants where microorganisms are not eliminated are open to the criticism that microörganisms in the cultures may be responsible for the formation of certain amounts of the gas.

Neller's results (46) further complicate an interpretation of studies of influences of plant development on production of carbon dioxide in soils, since they suggest that development of plants in soil causes an acceleration in the rate of transformation of the organic soil constituents.

Although there may be justification for the conclusion that root respiration is responsible for a large contribution to the carbon dioxide of soil air, there is fully as much reason for concluding that microörganisms about roots are responsible for a large part of the increase in carbon dioxide which accompanies the growth of higher plants. In this connection it may be sufficient merely to cite the results obtained by Lundegardh (41, p. 209) which show that as much as 45 per cent of the carbon dioxide formed about roots may arise from microbial action.

In view of the limited information which is now available concerning the importance of microörganisms as agents of formation of carbon dioxide in the zone of root development of higher plants, it is logical to delay speculations concerning the relative quantities of carbon dioxide contributed by microörganisms until more exact information is obtained.

EXPERIMENTAL PROCEDURE

The specific object of the following experiments was to determine what differences exist between the abundance and activity of microörganisms on the imme-

diate surfaces of plant roots, in soil close to the roots, and in soil farther removed from the zone of root development. This followed the anticipation expressed in a preceding publication (65) that, if it is assumed that root products are of major importance in determining the pronounced increases in microörganisms in the soil about roots, greater effects should appear at the root surfaces. These experiments were conducted during the months of June to October, 1929. It is interesting in this connection that the experiments of Gräf (14), which in some respects are similar to those to be reported, appear to have been performed simultaneously.

Two series of plants were used. One series consisted of second year growth of the three biennials, table beets, mangel beets, and sweet clover. Representatives of the same planting had been used during growth the first year for studies already reported (65, 66). In most cases three samples were taken about each plant as follows: (*a*) At 15 cm. distant from the main root of the beets and 30 cm. laterally from the base of the sweet clover to a depth of 12 to 15 cm.; (*b*) from the region of development of the roots of the plants to a depth of 12 to 15 cm.; (*c*) from the immediate root surfaces after all of the soil material which was readily shaken off had been removed. This last sample was obtained by scraping off the thin superficial layer of the root surface with a sterilized steel spatula. It consisted of both soil material clinging to the root surfaces and the outermost portion of the root tissue. These various samples were gathered about the plants at three periods during the summer. At these times the mangel beets and table beets had been planted for 375, 399, and 456 days. The sweet clover plants were 356, 380, and 437 days old at these periods.

The second series of plants consisted of a new planting of bush beans, mangel beets, and field corn. An attempt was made to confine the roots but at the same time create conditions which would permit free movement of materials between the region where the roots were confined and the remaining body of the soil. To accomplish this purpose, the seeds were planted within closely woven wire cylinders. The cylinders were prepared of 30-mesh tinned wire. They were sunk into the ground to the depth of 40 cm., projecting slightly above the surface. The cylinders were either 10, 15, or 20 cm. in diameter. The different sizes were used in order to accommodate the plants as they developed during the season. It was hoped that the wire would confine the roots so that samples of soil taken at various distances from the outside of the cylinders would represent soils occurring at certain regions away from the roots of the plants. By far the greatest portion of the roots was retained by the wire screen. However, the wire corroded somewhat, and many roots penetrated the screening even where it did not corrode. Consequently, the samples which were obtained did not represent soils free from roots in most cases but soils exposed to different degrees of root penetration.

The plants were well isolated from one another so that there was no danger of root systems from one plant penetrating the soil about the region of development of another. The samples were obtained by the following procedure. An

amount of soil was dug out, to the depth of about 17 cm., in the form of a circular trench just outside the region 30 cm. from the position of the wire cylinder. A thin layer of soil was taken from the entire cylindrical exposed surface, which was 30 cm. distant from the wire cylinder and 17 cm. deep. This was thoroughly mixed together and a representative portion was put through a 3-mm. sieve. A sample of about 1,000 gm. of sieved soil was brought to the laboratory for various determinations.

After the first sample was obtained, soil was removed up to the region 15 cm. distant from the wire cylinder. Here a second sample of soil was taken in a manner similar to the aforementioned. Then most of the soil surrounding the cylinder to a depth of 17 cm. was removed, leaving only a layer 3 cm. thick about the cylinder. This layer of soil surrounding the screening was removed, mixed, and sieved. This was sample 3.

The cylinder of soil was next removed, and the soil contained in the cylinder to a depth of 17 cm. was separated from the roots. This was sample 4. The roots were brought to the laboratory where they were scraped. The soil particles which were thus removed from the root surfaces, together with the superficial root tissues and rootlets were gathered. This was sample 5. Such samples of material were gathered about these plants at two periods during the summer, first, after the seed had been planted 66 days, and second, 113 days subsequent to planting.

All of the samples were examined for: (*a*) abundance of bacteria developing upon albumin agar, (*b*) abundance of *Bacillus radiobacter* and related forms developing on glycerol-nitrate-soil-extract agar, (*c*) abundance of total and mucoid colonies appearing on nitrogen-free mannite agar, (*d*) abundance of actinomyces developing on albumin agar, and (*e*) abundance of fungi growing on an acid medium. The procedures used were those followed previously (64, 65).

Determinations were also conducted on the soil samples for the evolution of carbon dioxide, the amounts of nitrate nitrogen produced from the soil nitrogen in 30 days, and the amounts of nitrate nitrogen formed in 6 days in the presence of added ammonium sulfate and calcium carbonate. These determinations were outlined previously (66). All the soils were brought to 25 per cent moisture at the beginning of these determinations.

The type of material represented in the samples obtained from the root surfaces was not adapted to these measurements of biological activity, being both limited in quantity and containing large amounts of root tissue.

The soil with which these experiments were conducted was a Sassafras loam. In reaction it varied between pH 6.0 and 7.2 during the season; most frequently the reaction was close to neutrality. The plants all made excellent growth.

The microörganisms would become very incompletely dispersed in the diluting liquid from the sample obtained from the root surfaces by simple shaking in water preliminary to plating. Consequently, the material was triturated in a mortar, aseptic precautions being observed. The weighed sample was added

to a sterile mortar. Sufficient sterile water was added to make a thin paste and the material was triturated with a sterile pestle until a smooth, finely ground

TABLE 1

Moisture contents of soil and plant materials

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT AT PERIOD OF SAMPLING		
		375 days	399 days	456 days
		per cent	*per cent*	*per cent*
Table beet	15 cm. from main root	19.8	23.1	18.8
Table beet	Close to main root	20.2	21.9	17.2
Table beet	Superficial layer of the root	....	52.0	22.8
Mangel beet	15 cm. from main root	20.1	21.5	19.5
Mangel beet	Close to main root	18.3	22.5	18.1
Mangel beet	Superficial layer of the root	....	51.6	37.1
Sweet clover*	30 cm. from main roots	16.3	20.5	16.4
Sweet clover*	Close to main roots	11.8	19.5	17.0
Sweet clover*	Superficial layer of the roots	....	32.7	24.1

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT AT PERIOD OF SAMPLING	
		66 days	113 days
		per cent	*per cent*
Bean	30 cm. from wire	....	20.3
Bean	15 cm. from wire	18.5	20.4
Bean	Close to wire	17.5	20.6
Bean	Close to main roots	17.6	20.0
Bean	Superficial layer of the roots	....	16.8
Mangel beet	30 cm. from wire	....	20.6
Mangel beet	15 cm. from wire	17.5	20.0
Mangel beet	Close to wire	16.4	18.3
Mangel beet	Close to main root	15.6	16.3
Mangel beet	Superficial layer of the root	20.2	23.5
Corn	30 cm. from wire	....	18.8
Corn	15 cm. from wire	13.9	17.7
Corn	Close to wire	14.2	15.8
Corn	Close to main roots	13.2	15.8
Corn	Superficial layer of the roots	27.5	19.2

* For sweet clover the sampling periods are 356, 380, and 437 days.

substance was obtained. This was transferred to a sterile flask with sufficient sterile water to make a known initial dilution. Although not all of the soil

samples were treated in this manner, it is believed that this did not appreciably affect the general nature of the results. This conclusion seems justified from the results obtained where the soils were triturated the same as the root samples. All of the samples taken the second period about the plants 113 days old were triturated. These results are in general agreement with the others.

The results on abundance of organisms are reported on the basis of numbers per gram of soil material as taken from the field. The moisture contents of these samples are presented in table 1.

EXPERIMENTAL RESULTS

Abundance of Microörganisms

The results of determinations of the abundance of bacteria are shown in table 2. It is quite apparent that the plants exert pronounced effects upon the development of bacteria. The most striking effect appears at the immediate root surfaces where the organisms are many times as abundant as in the soil surrounding the roots. The least effect was observed under beans at the 113-day period, where 3.6 times as many bacteria were found on the roots as in the soil close to the roots. The greatest effect was observed under sweet clover at the 437-day period. Here 120 times as many bacteria were found on the roots as in the soil about the roots. It is not surprising that large numbers of bacteria were found on or within the roots of legumes because of the well-established relationship of specific bacteria with these plants. That there is similar extensive development of bacteria on roots of all of the plants is of particular interest.

Averages of the effects of all the plants show that 24.8 times as many bacteria were found on the plant roots as in the soil close to the roots. Exclusive of the legumes, there were 12.1 times as many bacteria. The results of the legumes alone show 50.3 times as many bacteria on the root surfaces. Except for the abundance of bacteria on or within the root surfaces, there is nothing distinctive about the effects of the legumes. In general there were as many organisms in the soils about the roots of non-legumes as the legumes.

It is also apparent from the results in table 2 that bacteria are less abundant in soils at considerable distances from the region of origin of the roots. Under the second year growth of biennials, with only one exception, the soils about the roots show considerably greater numbers of bacteria than the soils 15 cm. from the main mass of roots. These effects appear fully as striking under the plants 113 days old, because more samples were taken at various regions from the location of the principal roots. In these cases, there is a progressive increase in the abundance of bacteria the nearer to the plants the soils were obtained. These specific effects of the different plants are more readily understood when it is explained that no root development was apparent at this period 30 cm. from the wire under either beans or mangel beets, but was noted at this distance under corn. There were a few roots in the soil supporting beans and mangel beets at a distance of 15 cm. from the wire.

The younger bean and mangel beet plants (66 days) showed little effect of root development on the bacteria except on the root surfaces. By reason of its more extensive root development at this time, corn produced more apparent

TABLE 2

Influence of distance from roots on total bacteria in soil

(Numbers at dilution of 1/1,000,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet	15 cm. from main root	18.6	23.2	22.6
Table beet	Close to main root	49.4	38.2	49.0
Table beet	Superficial layer of the root	458.4	737.4	485.0
Mangel beet	15 cm. from main root	22.8	15.6	16.2
Mangel beet	Close to main root	18.8	61.6	44.4
Mangel beet	Superficial layer of the root	604.6	462.8	583.0
Sweet clover*	30 cm. from main roots	18.0	31.2	16.2
Sweet clover*	Close to main roots	35.0	56.2	28.8
Sweet clover*	Superficial layer of the roots	887.4	3,637.8	3,470.0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm. from wire	.....	18.6
Bean	15 cm. from wire	17.4	32.8
Bean	Close to wire	20.8	36.2
Bean	Close to main roots	17.6	55.4
Bean	Superficial layer of the roots	662.4	199.4
Mangel beet	30 cm. from wire	.....	18.6
Mangel beet	15 cm. from wire	16.2	27.0
Mangel beet	Close to wire	14.8	33.4
Mangel beet	Close to main root	20.5	57.4
Mangel beet	Superficial layer of the root	92.4	427.4
Corn	30 cm. from wire	.....	22.8
Corn	15 cm. from wire	14.2	26.2
Corn	Close to wire	25.4	44.8
Corn	Close to main roots	122.0	93.2
Corn	Superficial layer of the roots	1,315.6	653.4

* For sweet clover the sampling periods are 356, 380, and 437 days.

effects. Roots of corn penetrated to the region of 15 cm. from the wire at this period. Roots of beans and mangel beets scarcely penetrated the wire screening at this time.

From these data it is quite reasonable to suppose that the organisms are most affected where there is abundant development of roots; where root development is relatively scant, few bacteria appear.

Similar but more pronounced influences of plants were observed upon the abundance of *B. radiobacter* and related forms. The results are shown in table 3. As in the case of the general bacterial population, the organisms were found in far greater abundance on the root surfaces than in the soils close to the roots. Considering all of the plants together, there were 45.3 times as many bacteria on root surfaces as in soils close to the roots. Exclusive of legumes, these bacteria were 11.2 times as numerous on the roots. With the legumes alone, 113.3 times as many of these bacteria were found on the roots as in the soil surrounding the roots. It is natural to expect that these organisms would be proportionally greater in or on roots of legumes than on roots of non-legumes since *Bacillus radicicola* is included in the group and undoubtedly made up a considerable portion of the bacteria developing from samples taken from legume roots. Nevertheless, it is of particular interest that very large numbers of organisms of the Radiobacter group occur on the surfaces of roots of non-leguminous plants. Their significance still remains undetermined but the fact that they were found, indicates that they were able to obtain the necessary food materials in the environment about the roots.

There is a pronounced decrease in abundance of these bacteria the greater the distance the soils occur from the region of most extensive root development. This decrease is considerably more apparent than with the general bacterial population. In the case of the soils under the second year of biennials there were 2.1 times as many total bacteria in soils about the roots as in soils 15 or 30 cm. from the main roots. However, there were 4.5 times as many bacteria of the Radiobacter group. In the case of the soils supporting the first year growth plants, 3.1 times as many total bacteria were found close to roots as 15 cm. distant. In the same soils, 29.3 times as many bacteria of the Radiobacter group were found close to the main roots. These results are in agreement with previous observations which indicated that proportionally small numbers of organisms of the Radiobacter group occur in soils free from plant development (63, 65, 14). It is logical to conclude that organisms of the Radiobacter group are affected by plant development proportionally more than is the bacterial population as a whole.

Organisms developing upon mannite agar showed response similar to that of the general bacterial population (table 4). The bacteria were far more numerous on the root surfaces than in soil surrounding the roots. These organisms were much less abundant in soils distant from the central root systems and, as a rule, the greater the distance from the regions of most extensive root development, the lower the numbers of bacterial inhabitants.

Table 5 presents results of determinations of the bacteria which produce mucoid colonies on mannite agar. These bacteria are undoubtedly much the same as those referred to previously as members of the Radiobacter group.

The numbers are somewhat higher, possibly because, on this medium, certain bacteria were able to develop which were inhibited by the crystal violet in the

TABLE 3

Influence of distance from roots on Radiobacter developing on glycerine-nitrate agar

(Numbers at dilution of 1/10,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet	15 cm. from main root	16.0	14.4	7.0
Table beet	Close to main root	21.4	16.2	14.0
Table beet	Superficial layer of the root	113.6	110.4	92.0
Mangel beet	15 cm. from main root	1.4	4.2	4.6
Mangel beet	Close to main root	15.6	12.8	12.0
Mangel beet	Superficial layer of the root	78.4	472.0	72.0
Sweet clover*	30 cm. from main roots	2.4	5.2	4.4
Sweet clover*	Close to main roots	14.4	43.2	23.2
Sweet clover*	Superficial layer of the roots	610.0	8,540.0	9,760.0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm. from wire	.....	5.2
Bean	15 cm. from wire	0.8	3.2
Bean	Close to wire	4.6	8.0
Bean	Close to main roots	11.2	19.6
Bean	Superficial layer of the roots	844.8	164.0
Mangel beet	30 cm. from wire	.....	6.6
Mangel beet	15 cm. from wire	1.4	11.4
Mangel beet	Close to wire	5.8	13.8
Mangel beet	Close to main root	7.4	63.6
Mangel beet	Superficial layer of the root	202.0	246.0
Corn	30 cm. from wire	.....	2.4
Corn	15 cm. from wire	2.4	4.6
Corn	Close to wire	17.6	10.8
Corn	Close to main roots	322.0	47.8
Corn	Superficial layer of the roots	1,412.0	476.0

* For sweet clover the sampling periods are 356, 380, and 437 days.

other medium. The general conclusions from the results of these determinations are practically identical with those reported for the Radiobacter group. Doubtless the bacteria were very much affected by plant development and were

particularly favored by conditions on the root surfaces where extensive multiplication of the bacteria took place.

TABLE 4

Abundance of colonies of bacteria on mannite agar developing from soils obtained at different distances from the main roots of plants

(Numbers at dilution of 1/1,000,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet	15 cm. from main root	18.0	24.0	8.8
Table beet	Close to main root	44.0	32.6	20.8
Table beet	Superficial layer of the root	358.4	560.0	201.2
Mangel beet	15 cm. from main root	19.3	21.0	13.8
Mangel beet	Close to main root	31.2	44.0	19.6
Mangel beet	Superficial layer of the root	556.2	396.0	228.2
Sweet clover*	30 cm. from main roots	22.8	16.8	11.8
Sweet clover*	Close to main roots	29.0	46.0	16.6
Sweet clover*	Superficial layer of the roots	935.4	3,028.0	2,864.0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm. from wire	.....	7.8
Bean	15 cm. from wire	4.8	13.2
Bean	Close to wire	4.2	11.6
Bean	Close to main roots	9.6	25.6
Bean	Superficial layer of the roots	351.8	98.4
Mangel beet	30 cm. from wire	.....	8.4
Mangel beet	15 cm. from wire	5.8	11.4
Mangel beet	Close to wire	10.4	20.4
Mangel beet	Close to main root	11.6	41.4
Mangel beet	Superficial layer of the root	54.2	130.8
Corn	30 cm. from wire	.....	13.2
Corn	15 cm. from wire	7.6	15.8
Corn	Close to wire	14.0	27.4
Corn	Close to main roots	91.4	55.6
Corn	Superficial layer of the roots	523.2	300.0

* For sweet clover the sampling periods are 356, 380, and 437 days.

As seen from table 6, the actinomyces represent a group of microörganisms which are affected to a very slight degree by plant development. There is

little suggestion of similarity between these results and those previously reported for the bacteria. The data for the first year plants show no influence of

TABLE 5

Abundance of mucoid colonies of bacteria on mannite agar developing from soils obtained at different distances from the main roots of plants

(Numbers at dilution of 1/100,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet	15 cm. from main root	10.4	8.2	2.6
Table beet	Close to main root	11.2	12.2	4.6
Table beet	Superficial layer of the root	416.0	472.0	214.0
Mangel beet	15 cm. from main root	4.2	5.6	2.0
Mangel beet	Close to main root	10.8	24.0	6.0
Mangel beet	Superficial layer of the root	594.0	316.0	232.0
Sweet clover*	30 cm. from main roots	4.4	6.6	2.4
Sweet clover*	Close to main roots	19.8	34.8	8.8
Sweet clover*	Superficial layer of the roots	1,680.0	11,320.0	4,040.0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm. from wire	.....	2.6
Bean	15 cm. from wire	1.2	4.2
Bean	Close to wire	1.2	3.8
Bean	Close to main roots	5.6	17.4
Bean	Superficial layer of the roots	209.2	89.2
Mangel beet	30 cm. from wire	.....	3.0
Mangel beet	15 cm. from wire	1.2	5.4
Mangel beet	Close to wire	2.0	8.8
Mangel beet	Close to main root	2.7	13.2
Mangel beet	Superficial layer of the root	80.4	83.0
Corn	30 cm. from wire	.....	2.4
Corn	15 cm. from wire	3.2	6.0
Corn	Close to wire	9.5	18.8
Corn	Close to main roots	58.6	24.6
Corn	Superficial layer of the roots	858.0	448.0

* For sweet clover the sampling periods are 356, 380, and 437 days.

root development on the actinomyces which can be considered to be significant. The older plants show some limited effects upon the organisms. Averaging the effects of the second year growth of the biennials, it is noted that there were

3.9 times as many actinomyces found upon the immediate root surfaces as in the soil close to the roots. The differences in abundance of actinomyces on

TABLE 6

Influence of distance from roots on abundance of actinomyces in soil

(Numbers at dilution of 1/1,000,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet	15 cm. from main root	5.8	6.6	6.8
Table beet	Close to main root	5.0	6.4	5.8
Table beet	Superficial layer of the root	16.0	18.6	21.0
Mangel beet	15 cm. from main root	4.8	8.2	8.8
Mangel beet	Close to main root	3.2	8.2	8.0
Mangel beet	Superficial layer of the root	16.2	21.2	29.0
Sweet clover*	30 cm. from main roots	6.2	4.8	5.8
Sweet clover*	Close to main roots	7.0	3.8	4.4
Sweet clover*	Superficial layer of the roots	2.8	10.2	50.0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm. from wire	....	7.6
Bean	15 cm. from wire	4.2	10.0
Bean	Close to wire	3.0	8.0
Bean	Close to main roots	5.8	6.2
Bean	Superficial layer of the roots	10.6	12.6
Mangel beet	30 cm. from wire	...	10.0
Mangel beet	15 cm. from wire	4.4	11.4
Mangel beet	Close to wire	3.4	10.4
Mangel beet	Close to main root	3.3	6.8
Mangel beet	Superficial layer of the root	2.8	10.6
Corn	30 cm. from wire	...	8.4
Corn	15 cm. from wire	5.2	11.8
Corn	Close to wire	6.0	8.8
Corn	Close to main roots	1.0	10.2
Corn	Superficial layer of the roots	2.4	8.6

* For sweet clover the sampling periods are 356, 380, and 437 days.

root surfaces and in soils about roots of these biennials are significant statistically.

It appears from these observations that actinomyces may be affected by

development of plant roots, but that the effects are readily detected only with plants in advanced stages of development and even these plants affect the

TABLE 7

Influence of distance from roots on abundance of fungi in soil

(Numbers at dilution of 1/10,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet	15 cm. from main root	21.0	25.0	23.0
Table beet	Close to main root	18.8	30.0	26.8
Table beet	Superficial layer of the root	66.0	46.8	64.2
Mangel beet	15 cm. from main root	14.0	23.0	17.2
Mangel beet	Close to main root	24.4	30.6	20.0
Mangel beet	Superficial layer of the root	74.2	114.0	47.2
Sweet clover*	30 cm. from main roots	23.8	18.2	21.6
Sweet clover*	Close to main roots	17.6	26.0	20.0
Sweet clover*	Superficial layer of the roots	40.4	82.0	212.0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm. from wire	.....	24.6
Bean	15 cm. from wire	16.6	21.6
Bean	Close to wire	21.2	20.0
Bean	Close to main roots	25.8	19.2
Bean	Superficial layer of the roots	40.9	55.2
Mangel beet	30 cm. from wire	.....	25.8
Mangel beet	15 cm. from wire	22.8	25.0
Mangel beet	Close to wire	28.2	25.8
Mangel beet	Close to main root	24.0	30.0
Mangel beet	Superficial layer of the root	55.0	156.0
Corn	30 cm. from wire	.....	29.6
Corn	15 cm. from wire	17.2	23.2
Corn	Close to wire	20.8	29.6
Corn	Close to main roots	25.6	49.6
Corn	Superficial layer of the roots	224.0	278.0

* For sweet clover the sampling periods are 356, 380, and 437 days.

actinomyces far less than the soil bacteria. The organisms are more numerous only on the root surfaces and do not show the influences of plant development at

appreciable distances from the region of extensive root development. Previous observations (65, 67) are in general agreement with these results.

Fungi were affected somewhat more than the actinomyces by plant development. In all cases reported in table 7, greater numbers of fungi developed from the materials taken from the root surfaces than from the soils obtained from the regions close to the roots. Averaging the effects of all the plants, it is noted that 3.9 times as many fungus colonies developed from the root surface samples. Although there seems to be a general indication that greater fungus development occurs in soil close to the region of principal root growth than in soil at distances from this region, the results are not statistically significant. Conclusions based upon plate counts of fungi are seldom completely satisfactory, since the amount of material which may be responsible for a fungus colony on a plate is so uncertain. It seems likely, however, that the results are sufficiently consistent to indicate that root development favors the growth of fungi although the effects of the fungi may not be pronounced in regions of the soil not penetrated by large numbers of roots.

Activities of microörganisms

The results concerning formation of carbon dioxide are shown in table 8. In general, they lead to the same conclusion as those recorded previously (66), namely, that root development favors formation of carbon dioxide of microbial origin. In every observation greater amounts of carbon dioxide were produced by soils obtained from the regions of principal root development than from soils obtained at any distances farther removed from the plants. The differences are greater with soils which supported growth of the older plants. This might be anticipated in view of the fact that greater amounts of root residues would be expected to be supplied to the soil organisms from such plants.

It is also apparent that, in soils supporting first year growth of plants, almost without exception, the greater the distance from the region of main root development, the smaller the amount of carbon dioxide produced.

It is unfortunate that no measurements could be made of the amounts of carbon dioxide produced by microörganisms upon the immediate root surfaces. It is in this region that one would anticipate that the greatest microbial activity would take place, since the organisms were many times more numerous than in the soil surrounding the roots. It seems logical to conclude that, since the activity of microörganisms, as indicated by evolution of carbon dioxide, correlates so well with the distribution of bacteria in the soils, carbon dioxide would have been produced in greater amounts by microbes per unit of soil material on root surfaces than from any of the other soil materials investigated and reported in table 8.

There is no doubt that the data do not indicate how much carbon dioxide was produced by the same soils previous to their removal from the field for laboratory study. Judging from the large amount of information available concerning the influence of disturbance of soil on biological activities (79, chapters 28

and 29), there is reason to believe that the carbon dioxide produced by the soils is greater than would have been produced if the soils had remained undisturbed. It seems unnecessary to suppose that this greatly decreases the value of the data. It is reasonable to conclude that a similar proportionality exists between the amounts of carbon dioxide produced by the various soil samples pre-

TABLE 8

Carbon dioxide evolved from soils obtained at different distances from plant roots

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
		mgm.	*mgm.*	*mgm.*
Table beet	15 cm. from main root	15.38	21.95	18.38
Table beet	Close to main root	22.55	26.98	22.30
Mangel beet	15 cm. from main root	13.25	15.40	14.08
Mangel beet	Close to main root	20.80	25.33	20.95
Sweet clover*	30 cm. from main roots	17.85	16.85	12.65
Sweet clover*	Close to main roots	26.80	24.13	18.03

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
		mgm.	*mgm.*
Bean	30 cm. from wire	....	9.7
Bean	15 cm. from wire	11.5	12.0
Bean	Close to wire	11.3	12.0
Bean	Close to main roots	12.5	15.1
Mangel beet	30 cm. from wire	....	11.2
Mangel beet	15 cm. from wire	9.4	13.6
Mangel beet	Close to wire	10.7	14.9
Mangel beet	Close to main root	12.0	18.2
Corn	30 cm. from wire	....	10.3
Corn	15 cm. from wire	14.0	15.2
Corn	Close to wire	14.5	15.2
Corn	Close to main roots	20.5	25.0

* For sweet clover the sampling periods are 356, 380, and 437 days.

vious to removal from the field and the amounts found during laboratory incubation. In other words, those soils showing low evolution of carbon dioxide in the laboratory may be considered to have produced small amounts of carbon dioxide in the field; the soils forming larger amounts of carbon dioxide under laboratory conditions probably evolved proportionally large amounts of

carbon dioxide in the experimental plots. Justification for this conclusion is found in the data previously discussed concerning the abundance of microorganisms in the various samples of soil materials. Those samples which contained a great abundance of microörganisms at the time they were obtained

TABLE 9

Nitrate formation from soil nitrogen during 30 days of laboratory incubation of soils obtained from different distances from plant roots

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
		mgm.	*mgm.*	*mgm.*
Table beet	15 cm. from main root	1.81	2.20	1.56
Table beet	Close to main root	2.82	2.66	2.12
Mangel beet	15 cm. from main root	1.61	1.77	1.42
Mangel beet	Close to main root	2.96	2.00	2.60
Sweet clover*	30 cm. from main roots	1.69	1.98	1.52
Sweet clover*	Close to main roots	4.52	3.22	2.49

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
		mgm.	*mgm.*
Bean	30 cm. from wire	....	0.87
Bean	15 cm. from wire	1.41	0.88
Bean	Close to wire	1.36	1.19
Bean	Close to main roots	1.27	1.25
Mangel beet	30 cm. from wire	....	1.47
Mangel beet	15 cm. from wire	0.97	1.46
Mangel beet	Close to wire	1.05	1.76
Mangel beet	Close to main root	1.77	1.83
Corn	30 cm. from wire	....	0.76
Corn	15 cm. from wire	1.16	1.26
Corn	Close to wire	1.05	1.52
Corn	Close to main roots	2.07	1.95

* For sweet clover the sampling periods are 356, 380, and 437 days.

from the field gave evidence of proportionally high carbon dioxide production upon being incubated in the laboratory. The samples having small numbers of microörganisms showed low biological activity as measured by the formation of carbon dioxide.

Further evidences of increased biological activity in soils about roots is pre-

sented in table 9. These data indicate that the organic nitrogen of soils obtained from regions of extensive root development is more susceptible to rapid change to nitrate-nitrogen than the organic nitrogen contained in soils which support less root development. Almost without exception, the soils obtained

TABLE 10

Nitrate accumulation in 6 days from the oxidation of ammonia added as ammonium sulfate to soils obtained at different distances from plant roots

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
		mgm.	*mgm.*	*mgm.*
Table beet	15 cm. from main root	6.00	6.41	8.48
Table beet	Close to main root	8.20	7.70	9.36
Mangel beet	15 cm. from main root	5.83	6.00	7.68
Mangel beet	Close to main root	7.40	8.16	9.58
Sweet clover*	30 cm. from main roots	7.00	5.50	7.51
Sweet clover*	Close to main roots	7.30	8.43	9.47

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
		mgm.	*mgm.*
Bean	30 cm. from wire	....	7.03
Bean	15 cm. from wire	7.34	7.59
Bean	Close to wire	8.05	7.59
Bean	Close to main roots	8.55	7.63
Mangel beet	30 cm. from wire	....	8.65
Mangel beet	15 cm. from wire	7.16	7.69
Mangel beet	Close to wire	7.27	7.65
Mangel beet	Close to main root	6.73	8.76
Corn	30 cm. from wire	....	8.11
Corn	15 cm. from wire	9.30	8.57
Corn	Close to wire	9.43	10.77
Corn	Close to main roots	9.80	9.89

* For sweet clover the sampling periods are 356, 380, and 437 days.

from regions of greatest abundance of roots showed most nitrate accumulation. With the young plants (66 days), the favorable effects are not as consistent as they were with plants in more advanced development.

It is quite possible that similar rapid transformation of the nitrogenous substances occurs in the soils under field conditions. At least, it is logical to sup-

pose that the soil constituents required by nitrifying bacteria are present in proportionally great abundance in soils close to roots.

The data in table 10 suggests that the nitrifying population in the soil about the roots is able to transform ammonium nitrogen to nitrate-nitrogen somewhat more rapidly than the population of other soils. As with some of the other evidences of influences of plants on soil organisms, the older plants brought about the most pronounced effects. In every instance under second year growth of biennials, greater nitrification was observed in soils close to the main root systems. The results were not quite as consistent under the younger plants, but in general showed the greatest effects of the plants in the regions of most extensive root development. It may be concluded that either the nitrifying organisms in these soils are more efficient transformers of ammoniacal nitrogen or that these soils support a greater abundance of the bacteria.

DISCUSSION

A statement that roots of higher plants and cells of microörganisms exist together in soils would probably remain unchallenged, but various speculations as to the intimacy of relationships between soil microörganisms and root systems might be ventured. With information based upon the results just presented, upon those reported in the preceding papers of the series, and upon the related information gathered together in the following pages, conclusions will be advanced concerning these relationships.

In the body of the soil through which plant roots develop, there is a host of microörganisms having the capacity of attacking and transforming a great variety of organic materials. The region between the interior of root tissues and the soil appreciably removed from root surfaces is of particular interest as regards the activity of microörganisms. In this zone there appear marked changes in the organisms, these changes being initiated by the plants and undoubtedly affecting plant development. The term *rhizosphere* was applied by Hiltner to this portion of the soil where the microbial population is subjected to the effects of plant roots. It was Hiltner's idea that the bacteria develop as a result of root excretions. The formations consisting of bacteria on the root surfaces and within the roots were called *bacteriorrhiza* (17). It was considered that each plant favors the development of those bacteria which favor its nutrition. Accordingly, not only each plant group, but each plant, has different root excretions creating a reciprocal relationship between the plant and the bacteria. With changing plant nutrition there is an alteration in the relationship of the rhizosphere flora.

Theories concerning organisms in the rhizosphere have frequently centered about the idea that the relationships of microörganisms to plant roots are beneficial and that the bacteria are specific in their action. Caron (4) believed that, by inoculating soils with bacteria obtained from soils supporting good plant development, he could obtain increased growth of plants. By the use of bacteria isolated from the rhizosphere of beets, for the inoculation of soil,

Hiltner (20) claimed to have obtained increased plant growth. The inoculation of soil with Azotobacter has also been assumed to improve microbial conditions in the rhizosphere (28). Joshi (27) reported that he obtained increased amounts of nitrogen in non-legumes following inoculation of soil with nitrogen-fixing bacteria. Kostytchew (31) observed more favorable development of tobacco in soils receiving additions of cultures of Azotobacter. Truffaut and Bezssonoff (72) claim to have been able to grow corn to maturity with nitrogen-fixing bacteria furnishing the entire supply of nitrogen to the plants. The removal of soluble nitrogenous materials from soil at certain stages of plant development may partly explain the increase in nitrogen-fixing bacteria which has sometimes been observed.

Hiltner (17) conceived the idea that the bacteriorrhiza formations prevent penetration of roots by injurious organisms. More recently, E. Hiltner (16) extended the idea, ascribing to the bacteria the rôle of acting as a filter, protecting the plants against the absorption of injurious minerals.

Since mustard and certain other cruciferous plants are able to develop well in soils apparently deficient in nitrogen, it has been considered that these plants create conditions particularly favorable for the development of Azotobacter in the rhizosphere and that the plants are able to benefit from nitrogen which is fixed by these bacteria (18, 19, 21, 5, 6, 7, 78, 54, 37, p. 20). However, it is not a property specific to cruciferous plants to favor development of Azotobacter. Poschenrieder found Azotobacter generally distributed on roots of a great variety of plants (55).

Stoklasa (68) found that the soil about roots supports an extremely varied assortment of microörganisms, including spore-formers and non-spore-formers, Azotobacter, ammonifiers, denitrifiers, yeasts, and molds. It was believed that the mucilaginous material produced by many of the organisms exerts colloidal effects quite favorable to plant development. Gottheil (13) found many organisms about roots and those he found appeared to be very generally distributed. These observations led him to conclude that it is unlikely that the bacteria play specific rôles about particular plants. Truffaut and Vladykov (73) found a great variety of microörganisms about roots of wheat. Löhnis (37) was of the opinion that differences in the types of organisms existing on the roots of different plants were not as great as some investigators had concluded. Even the differences in types of organisms on roots of cultivated and wild plants did not appear to be pronounced. Lundegardh considered that the influences of microörganisms upon formation of carbon dioxide about roots could be best explained by assuming that roots are surrounded by a film of soil bacteria which are actively respiring (41).

Although incomplete, this review indicates that certain particularly important rôles have been ascribed to the organisms occurring about roots of plants. Even with our present limited knowledge of the facts, some of the opinions appear to be highly speculative. Although it is reasonable to suppose that the microbial populations about roots of all plants are not qualitatively alike,

there is no justification for assuming that plants favor the development of only those organisms that are beneficial.

The surprisingly extensive development of bacteria on root surfaces leaves little room for doubt as to whether or not roots supply food material for the development of bacteria even when the roots are apparently healthy. There is considerable direct evidence and other indirect evidence concerning the food substances. From the fact that associative growth of legumes and non-legumes is frequently advantageous to the non-legumes, Lipman concluded that nitrogenous materials are obtained from the legumes either as a result of decay of roots or the elimination of soluble materials from the roots into the surrounding soil (35, 36). Mazé (43) noted the presence of organic substances in nutrient solutions supporting growth of plants. Under more carefully controlled conditions, such elimination of organic materials was found by Lyon and Wilson (42). Minima (45) presented evidence which led to the conclusion that organic acids are excreted from roots in considerable amounts during periods of advanced vegetative development. It was further noted by Sabinin and Minina (61) that the plants which assimilate phosphorus from tri-calcium phosphate excrete more organic acids than the plants which utilize less of this phosphate. Demidenko (10) found considerable organic substances excreted into culture solutions by tobacco and corn. It is of particular interest that he observed that the organic compounds are given off in large quantities up to the time when the assimilation processes reach a maximum. Microbial activity also follows a similar course of change, being greatest at periods of advanced vegetative development or fruiting (65, 66, 67).

Legumes are believed to favor development of symbiotic nitrogen-fixing bacteria about their roots previous to root penetration, through the excretion of amounts of certain organic substances from the roots. Slimy material is frequently excreted about root hairs of many plants other than legumes (80).

In addition to materials excreted by plant roots, certain root parts are regularly sloughed off (80). These include root caps, root hairs, and epidermal cells separated from the roots with advance in age. Information concerning differences in the substances coming from roots of different plants during various periods of growth is not yet available. No doubt it will be shown that the differences are sufficient to affect various elements of the soil population in different ways.

Even greater portions of the roots may be appropriated by soil microörganisms during plant development. Many of the roots which initiate growth, die before the plant is mature, due to loss of vigor brought about by any of a number of soil conditions (80). Decrease in moisture supply may result in the death of roots near the surface; a rise in the water table may cause the death of deep roots. The subterranean portion of the plant continually undergoes changes; the root system extends, loses certain portions, and rebuilds lost parts upon modifications in conditions of temperature, moisture, reaction, composition and movements of gases in the soil (3). The soil conditions are par-

ticularly important as affecting activity of plant pathogens (23, 24, 11, 25, 26, 79, chap. 30). In some cases the environment modifies infection by affecting the parasite, as in onion smut, where temperature affects spore germination. In other cases, infection is determined by the environment modifying the host, creating lowered resistance of the plant at either high or low temperatures moisture contents, or reactions. The resistance may be related to thickness and composition of cell walls or other effects. It has been shown by Thornton (71) that even the symbiotic development of legume bacteria may change to parasitism when conditions lead to decreased formation of carbohydrates within the plant. Rayner and Smith (57) refer to an observation that young roots of Calluna were found surrounded by an extensive development of bacteria in soils somewhat unfavorable to development of the plant. As a normal sequence of events, the attack of plant pathogens makes conditions favorable for the attack of certain remaining portions of roots by the saprophytic soil organisms.

It thus seems apparent that there is justification for the conclusion that sufficient organic substances come from roots to favor greatly the development of microörganisms about roots during plant development.

Organisms possessing great differences in capacity to attack plant tissues inhabit soils, most of the organisms being strictly saprophytic, however. There are several well established cases of root penetration by microörganisms, as with the legume bacteria and mycorrhiza fungi. Accompanying the specific legume bacteria, there is very frequently an organism called *B. radiobacter* (39, 34). Evidence also points to the fact that certain non-legumes may be quite generally penetrated by specific bacteria (79, chap. 4 and 62). A host of plant pathogens gain entrance to tissues. All of these cases are related to the characteristics of specific microörganisms, and such associations of microörganisms and plants are not common to the great majority of the soil population.

Root tissues which appear to be vigorous may not be entirely free from microorganisms. Jones (26) found fungi quite generally invading plant roots. Bacteria other than plant pathogens have also been detected frequently in roots. Perotti and his associates have found bacteria in the roots of a great variety of plants (47, 48, 49, 52, 53, 50, 51, 77). They were found diffused in the cortex and in the intracellular and intercellular spaces but not within the vascular system. In about 70 per cent of the cases studied, bacteria were found. It has not been assumed that the organisms are of pathogenic significance. It was assumed by Lominsky (40) that animal pathogens could gain entrance through wounds in plants and live within the tissues and also that all organisms penetrate root tissues. These results could not be duplicated by others (60, 8, 29), but Russell (60) observed that, subsequent to inoculation of plants with bacteria, many of the saprophytic forms persisted for a considerable period of time and even scattered some distances from the points of inoculation. Apparently some non-pathogenic bacteria can gain entrance into root tissues and persist in this environment, although most indications point to the fact that

only small numbers of bacteria occur in root tissues which have not degenerated.

A survey of these various observations leads to what seems to be a natural conclusion concerning some of the relationships between plant roots and soil organisms. Knowing that microörganisms develop where the supply of organic materials and environmental conditions are favorable, the development of organisms about roots seems to be controlled principally by the supply of organic matter. The sloughed off root caps and root hairs supply considerable food as the roots grow into the soil. Some amounts of exudations may appear at various regions of the root systems. Some cortical cells on growing roots may be sloughed off and superficial cells may become invaded by soil microorganisms. During a great portion of the period of plant development, most soil organisms are undoubtedly limited to regions outside of the roots. In response to organic materials coming from plant roots the microörganisms reach their maximum development in periods of advanced plant growth preceding extensive degeneration. Certain organisms may penetrate beyond the external cells, the depth of penetration being determined by the vigor of the root cells and the nature of the organisms in the rhizosphere. Some organisms may enter through openings in the superficial tissues.

The soil organisms should not be considered as passive individuals waiting for the death of roots before attack. They consume whatever materials are available at any time, the availability of root substances being affected by the resistance of cell membranes to microbial penetration and the occurrence of materials inhibitive to their development.

At the time of maturity and degeneration of the vegetative structures, many changes appear in the roots. As assimilation within the plant decreases, the root tissues become weakened through reduced food supply and they become the prey of soil organisms. The root hairs and fine roots disappear and the tissues of the larger roots become more deeply penetrated by microorganisms. The root system degenerates progressively until, at the time when the superficial portion of the plant is dead, there is little of the original root system which is in a vigorous condition free from microörganisms. All of this sequence of events may be greatly modified by particular effects such as extremely unfavorable environmental conditions or invasion of pathogens which may involve the entire root system.

Complete knowledge concerning the physiology and cytology of root systems during the course of growth, maturity, and degeneration will aid materially in explaining the changes which take place in microbial development. It is unlikely that observations at a single period would indicate the nature of the effects exerted by a plant, since the reactions of the microbial population undoubtedly closely follow the physiological changes in the root system.

SUMMARY

Experiments were performed to determine what differences exist between the abundance and activity of microörganisms on the immediate root surfaces of

plant roots, in soil close to the roots, and in soil farther removed from the zone of root development. The results may be summarized as follows:

Microörganisms were much more abundant about roots. The most pronounced increases were noted in the region of the root surfaces where the organisms were many times as numerous as in the soil close to the roots.

The general bacterial population and the bacteria of the Radiobacter group were affected by root growth to a much greater extent than the actinomyces and filamentous fungi.

Greater numbers of bacteria were found on roots of legumes than on those of non-legumes but the non-legumes exerted marked effects on the organisms. No characteristic effect of legumes was observed except in the region of the root surfaces. As many organisms were found in soils about roots of non-legumes as in soils about roots of legumes.

In general, the greater the distance from the region of extensive root development the smaller the number of bacterial inhabitants found in the soil. Effects of roots on fungi and actinomyces were noted only in materials obtained from the root surfaces.

Greater amounts of carbon dioxide were produced from soils obtained from regions of extensive root development than from soils obtained at any distance farther removed from the plants. A close correlation appeared between the amounts of carbon dioxide produced by the soils and the abundance of bacteria detected in the samples.

Nitrification was most active in soils obtained from regions of maximum root development.

An explanation of the relationships between soil microörganisms and plant roots is advanced, which is based upon these observations, those presented in previous reports of this series of papers, and numerous reports of other related material.

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SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: V. EFFECTS OF PLANTS UPON DISTRIBUTION OF NITRATES

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The nitrate ion in the soil solution commands particular attention because it is of primary importance in the development of most cultivated plants. It frequently occurs in soil in amounts insufficient for maximum plant growth and is supplied in the form of fertilizers only at considerable expense and labor. The fact that nitrate may be the end product of certain bacterial reactions, and is further susceptible to other modifications by soil organisms complicates the study of the availability of the nitrogenous resources of the soil for plant development.

Since nitrates generally originate through biological action, the amounts of nitrates in soils are frequently considered to reflect the degrees of microbial activity in these soils. There is also little question that in humid regions the soils that are rich in nitrates are among the most fertile. Burd (3) considered that the nitrate content of uncropped soils was the most valuable single criterion for appraising their crop-producing power.

There are, however, so many factors which affect the nitrate content of soils that isolated determinations in uncropped soils and in soils planted to different crops at various stages of development give no true representation of either the biological activity, the rate of nitrate formation, or the capacity of the soils to support plant growth. Stewart (24) found no nutrient ion in the soil solution affected more by cropping than was nitrate. In the soil solution of unplanted soil, nitrate was more abundant than any other nutrient; other plant nutrients as well as nitrate were lowered by cropping but nitrate more than any other.

Influence of plants upon soil nitrates.—The depression in nitrate content due to plant growth has been repeatedly observed and needs little comment other than mention of the fact that differences have been observed under different plants and the same plant at different stages of development (3, 4, 6, 7, 8, 9, 13, 14, 17, 24). Most of the removal of the nutrient ions occurs during the stages of extensive vegetative development when the plants make their greatest absorption of nutrients (10). During the late stages of development, many plants decrease in mineral nutrients and apparently a portion of the previously absorbed substances returns to the soil (4). Subsequent to death of the plants the nitrate content of soils tends to rise again (4, 24).

There are differences in the effects of specific plants upon soil nitrates. King and Whitson (12) considered that plant growth favored nitrification since the sum of the nitrogen in the crop and the nitrate of the soil supporting the crop was greater than the nitrogen in the nitrate of fallow soil. Russell (17) found that during late summer and early autumn the fallow land was richer in nitrate even after allowing for the nitrate taken up by the crop. Certain plant characteristics observed by Lyon, Bizzell, and Wilson (13, 14, 28) could be interpreted to explain these differences. It was observed that grass and wheat tended to depress nitrate accumulation whereas corn favored nitrate formation during the early stages of development. The nitrate content of soils previously grown to legumes maintained a higher level than the nitrate content of soils supporting certain non-legumes. The composition of the organic residues of these plants varied greatly and it seems likely that the specific differences exerted by the plants were due to a large extent to the differences in the plant residues. These residues were decomposed by microörganisms and if they contained appreciable amounts of nitrogen the result was favorable to the processes leading to nitrate accumulation. If the residues were low in nitrogen the assimilation of nitrogen by the microörganisms was sufficiently great to cause a depression in the rate of nitrate formation.

It seems likely, therefore, that at least two factors may be active in lowering nitrate concentration about roots: the absorption by plants and the absorption by microörganisms, the extent of the second being determined by the amount and nature of the organic materials put at the disposal of the microörganisms by the plant.

Nitrates in fallow soils.—Where plants are not developing, there are numerous factors exerting marked effects upon the distribution of nitrates. Temperature, rainfall, soil texture, amount and composition of the soil organic matter, soil reaction, and abundance of inorganic soil constituents are determining factors (1, 17). Almost invariably nitrates accumulate in fallow soils, and part of the increased fertility resulting from fallowing is to be ascribed to this change. The course of change in amount of nitrates in a fallow soil is not entirely in one direction, however. The fact that nitrates enter solution readily and are not appreciably retained by the soil colloids explains why nitrates migrate extensively in soils. Under the influence of heavy rainfall, nitrates pass into the deep regions of the soil and upon such occasions the quantity of nitrates may be greater in the subsoil than in the surface soil. Under conditions of rapid evaporation at the surface the nitrates may again rise with the capillary water (7, 8, 18, 19). One of the important rôles played by cover crops is the absorption of nitrates from the soil solution during periods when they might otherwise be lost from the soil in leaching waters, if no plant cover existed.

Variability in distribution of nitrates in soils.—These various factors and probably others create a lack of uniform distribution of nitrates in soils. Waynick (26) observed a pronounced variation in nitrate content in soils even

lacking vegetation. In 81 samples of surface soil taken from an area 50 feet in radius he observed variations from 1.0 to 4.5 mgm. of nitrate nitrogen per 100 gm. of soil. The 81 samples of subsoil varied from 0.3 to 2.2 mgm. Prince (16) observed similar degrees of variation. The variation was greater in soils which had been treated with animal manure than in untreated soils. More recently Thornton and Gray (25) noted that the nitrate content of unplanted soil not only is different from place to place but changes within very short intervals of time. Within 2 hour intervals such extreme changes were observed as from 35 to 14 p.p.m. in one case and from 26 to 50 p.p.m. in another. There was even some suggestion of diurnal fluctuations.

The degree of variability created by plant development seems to have commanded little attention although it is acknowledged that considerable modification may be created by plants. Hoagland (11) states the following:

> It is, perhaps, not entirely accurate to picture the soil solution of an entire mass of soil gradually becoming reduced in concentration. . . . . Considering the plant as a whole, however, the supply of mineral elements would decrease as growth proceeds, if the mass of available soil were limited in amount, or if root growth ceased.

In unlimed soil, Blair and Prince (2) found more nitrate nitrogen at a distance from the corn plant than near the base of the stalk. In limed soils little difference was noted. It was concluded that the difference between the limed and unlimed soils was due to the more limited root systems of the plants in the unlimed soils.

EXPERIMENTAL PROCEDURE

The results reported herein are concerned with the degree to which plant development affects the distribution of nitrate nitrogen in soils. Soils which were used for studies of the influences of plant development upon the soil microorganisms (20, 21, 22, 23) were observed for nitrate content. The determinations were made over a period of 2 years. During the first year comparisons were made between the contents of nitrate nitrogen in soil about plant roots and in soil supporting no plant development. Certain biennials of this planting were continued for study during the second year and a newly planted crop was also used. Determinations were carried out on soils about these plants at different distances from the region of massive root development. Nitrates were determined by the phenoldisulfonic acid method, and the results are reported on the basis of the dry soil.

EXPERIMENTAL RESULTS

The results shown in tables 1, 2, and 3 show quite definitely that the nitrate nitrogen was much less abundant in soil taken from the vicinity of root development than in soil devoid of roots. These experiments were conducted in the field and consequently the numerous climatic factors as rainfall, evaporation, and fluctuations in temperature exerted their uncontrolled influences on

the soils. There appears to have been no regular course of change in the nitrate content of the unplanted soil during the first year but in the second year (see table 3, periods of 375, 399, and 456 days) there was a progressive increase in nitrate content. The number of observations is, however, too limited to be of appreciable importance regarding the seasonal change in nitrates in unplanted soil.

In tables 1 and 2 lines are drawn between the columns of data to indicate the division between living and dead plants. The potato plants died between the sixty-third and eighty-sixth day of sampling, the oats between the eighty-

TABLE 1

Influence of root development of potatoes, oats, and beans upon nitrate content of soil

PERIOD FROM TIME OF PLANTING	NITRATE NITROGEN IN SOILS—MGM. PER 100 GM. OF SOIL						
	Unplanted	Under potatoes	Loss due to growth of potatoes	Under oats	Loss due to growth of oats	Under beans	Loss due to growth of beans
days							
44	0.76	0.53	0.23	0.41	0.35	0.41	0.35
63	1.74	0.46	1.28	0.55	1.19	0.40	1.34
86	1.14	1.64	−0.50	0.83	0.31	0.69	0.45
138	0.91	1.86	−0.95	1.13	−0.22	0.62	0.29
173	1.00	1.23	−0.23	0.62	0.38	1.03	−0.03

TABLE 2

Influence of root development of corn and rape upon nitrate content of soil

PERIOD FROM TIME OF PLANTING	NITRATE NITROGEN IN SOILS—MGM. PER 100 GM. OF SOIL				
	Unplanted	Under corn	Loss due to growth of corn	Under rape	Loss due to growth of rape
days					
44	0.76	0.64	0.12	0.44	0.32
63	1.74	0.37	1.37	0.53	1.21
86	1.14	0.41	0.73	0.41	0.73
138	0.91	0.55	0.36	0.66	0.25
173	1.00	0.52	0.48	2.13	−1.13

sixth and the one-hundred and thirty-eighth day. The beans and corn were killed by frost just previous to sampling on the one-hundred and seventy-third day. It is quite clear that the roots of the potato plant exerted no depressing effect after death; a pronounced increase in nitrate is observed however at these last periods. The effects of the other plants are not consistent. In the 42 periods of study of soils during which plants were living there are only two periods where there was less nitrate in the unplanted soil.

It is not possible to draw definite conclusions regarding the periods of greatest removal of nitrate. Since the soils were not in closed containers the amounts

of nitrate in the unplanted soil do not indicate the amounts of nitrate that were formed; appreciable quantities were undoubtedly moved about outside of the region of sampling. It is interesting, however, that most of the plants were in the stage of extensive vegetative development at the sixty-third day period, the time when the maximum difference between unplanted and planted soils appeared during the first year. The sweet clover plants were comparatively small at this stage. The lowest nitrate content under this plant occurred at the 173-day period, which was the period of greatest vegetative growth. Much more regular changes in response to plant development would undoubtedly be noted if soils were confined in closed containers.

Table 4 records observations made in soils about three biennials during their second year of development. It is apparent that the nitrates in soils supporting no plants are consistently more abundant than in soils either close

TABLE 3

Influence of root development of table beets, mangel beets, and sweet clover upon nitrate content of soil

PERIOD FROM TIME OF PLANTING	NITRATE NITROGEN IN SOILS—MGM. PER 100 GM. OF SOIL						
	Unplanted	Under table beets	Loss due to growth of table beets	Under mangel beets	Loss due to growth of mangel beets	Under sweet clover*	Loss due to growth of sweet clover
days							
44	0.76	0.41	0.35	0.47	0.29	0.40	0.36
63	1.74	0.38	1.36	0.32	1.42	0.70	1.04
86	1.14	0.58	0.56	0.51	0.63	0.59	0.55
138	0.91	0.39	0.52	0.46	0.45	0.64	0.27
173	1.00	0.34	0.66	0.17	0.83	0.08	0.92
375	0.69	0.53	0.16	0.83	−0.14	0.28	0.41
399	1.88	0.54	1.34	0.57	1.31	0.57	1.31
456	3.11	0.93	2.18	1.09	2.02	1.20	1.91

* For sweet clover the sampling periods are 25, 44, 67, 119, 154, 356, 380, and 437 days.

to the roots or a short distance from the main root systems. The differences between the soils 15 cm. distant and those close to roots are not consistent, suggesting that the absorption systems of the plants kept the nitrate content to a fairly low level in these zones even during the second year of development. In all three cases, at the time of the final sampling, nitrates were more abundant adjacent to the roots than a short distance away, possibly a significant change coinciding with degeneration and decomposition of root materials near the end of their extended development over 2 years.

The results in table 5 are more definite and informative, since more regions of sampling are involved. In the cases of mangel beets, differences are apparent at the three regions of sampling at the 66-day period. At the 113-day period the root system created similar degrees of removal to the distance of 8

TABLE 4

Distance through which root development exerts its effects upon the nitrate content of soils—second year growth of biennials

PERIOD FROM TIME OF PLANTING	NITRATE NITROGEN IN SOILS—MGM. PER 100 GM. SOIL		
days	Unplanted	15 cm. from main root*	Close to main root
	Table beets		
375	0.69	0.59	0.53
399	1.88	0.57	0.54
456	3.11	0.52	0.93
	Mangel beets		
375	0.69	0.57	0.83
399	1.88	1.11	0.57
456	3.11	0.79	1.09
	Sweet clover		
356	0.69	0.20	0.28
380	1.88	0.50	0.57
437	3.11	0.96	1.20

* For sweet clover the soil was sampled at 31 cm. distant from the main root system.

TABLE 5

Distance through which root development exerts its effects upon the nitrate content of soils—first year of a planting

PERIOD FROM TIME OF PLANTING	NITRATE-NITROGEN IN SOILS—MGM. PER 100 GM. SOIL			
days	40 cm. from main root	23 cm. from main root	8 cm. from main root	Close to main root
		Mangel beets		
66		4.36	3.60	3.20
113	1.20	0.44	0.35	0.38
		Beans		
66		2.59	1.55	0.89
113	1.80	1.09	0.94	0.63
		Corn		
66		1.37	0.50	0.67
113	1.37	0.91	0.36	0.56

cm. from the roots and greatly lowered the nitrates at a distance of 23 cm., but much higher quantities were found at 40 cm. distant.

The more limited root system of the bean plants brought about less disappearance of nitrates at a distance from the region of the origin of the roots. In both periods there is a progressive decrease in nitrates the nearer the soil is to the central root system. However, the plants appear to exert an influence to the distance of 23 cm.

The effects of corn are quite pronounced at a distance of 8 cm. at the first period and definitely extend to the distance of 23 cm. at the second period. The great variation in root systems of different plants undoubtedly modifies the distribution of nitrates in different ways. The extent of the root systems varies, the amounts of absorption at different periods are unlike, and the quantity and quality of the root residues create different responses in microbial activity.

DISCUSSION

It seems evident that plant development may greatly modify the distribution of nitrate nitrogen in soil, at least under certain conditions of plant growth. Where plants are quite completely isolated from one another the root systems fail to bridge the gap between and there develop zones from which extensive removal takes place through absorption by roots and other agencies. At a distance from these regions there is less removal by plants. A more dense stand of plants would tend to eliminate this variation in the lateral distribution, since the network of roots would permeate the soil quite completely at the advanced stages of development. The density of the stand that would bring nitrates to a somewhat uniformly low level would depend upon the extent of the absorptive system, which in turn would differ with the stages of development of any one plant, would vary with different kinds of plants under any one set of conditions, and would further be altered by climatic and soil conditions.

Accompanying the influence of root absorption there is the influence of microbial absorption of nitrates about roots during the decomposition of root residues and exudation products (14). These effects are presumably not the same under all plants or at different periods of development of a single plant. At certain periods at least, root absorption is much the more important factor. The root residues of sweet clover should presumably be somewhat richer in nitrogen than the residues of certain of the non-legumes which were used. However, the lowest content of nitrates in soils was observed under the sweet clover at the stage of its maximum vegetative development (173 days).

The vertical distribution of nitrates is undoubtedly also affected by plant development. In humid regions the effects at appreciable depths might not be as pronounced as the effects near the surface, since nitrates are concentrated in the superficial regions. In semi-arid regions the changes at lower levels might be more striking.

The observations of Blair and Prince (2) indicate that treatment of soil with lime so modified root development that more complete nitrate absorption took

place. From the results of Weaver, Jean, and Crist (27) it is very apparent that root systems are susceptible to great modifications by fertilizer treatments, differences in texture and structure of soils, as well as differences in amount and distribution of moisture. These numerous factors affecting root development undoubtedly lead to effects upon the removal of nitrates from the soil.

The decrease in nitrate concentration about roots can be interpreted as indicating that certain other ions are likewise depleted (3, 4, 5, 6, 24). Exception may appear with phosphate and bicarbonate, the phosphate occurring in low concentrations whether or not plants develop and the bicarbonate definitely increasing about roots (6, 15). The final balance as a result of plant growth shows a pronounced decrease in electrolytes (10). It seems likely that such disappearance of nutrient ions would depress microbial activity, since it is generally conceded that the addition of fertilizer salts to soils of humid regions favors microbial development. However, one cannot conclude that, since nitrate concentration is lower about plant roots, microbial development is also decreased. In fact, very pronounced favorable effects are exerted upon the growth and activity of the soil microbes (20, 21, 22, 23). It seems justifiable to conclude, therefore, that some other effect than the change in nitrate concentration exerts the dominant influence upon the microörganisms to cause their increased development. It has been previously concluded that organic exudations from roots and degenerating root parts are of major importance. Other changes such as modified circulation of air and moisture probably lead to some of the responses in the organisms.

Likewise, the proportional amounts of nitrates about roots of developing plants compared with the amounts in soils at a distance from roots give no suggestion of the rates of nitrification in these soils or the potential rates with which nitrates would accumulate if the plant factor was eliminated at any time. In fact, in many instances, nitrate formation progresses more rapidly in soils taken from the root zone than in soil devoid of roots even though the soil which supports the roots contains very small amounts of nitrates at the time the soils are removed from the field (21, 22, 23).

It may be concluded that the amounts of nitrates in soils supporting plant development are of little use as indexes of either the activity of the soil organisms or the fertility of the soils. Plant growth as well as other agencies may create great variations in the distribution of nitrates in soils, and consequently [in the words of Burd (5)]" . . . . the large variation in nitrate content frequently observed in samples drawn within short distances of one another in the field may be without significance in terms of crop production."

SUMMARY

Determinations of the abundance of nitrates in soils about roots of developing plants and in soils free from roots indicate that, under field conditions, plants greatly lower the nitrate content of soils.

The degrees of removal of nitrates differ with the stages of growth of the plants.

The nitrate contents of soils at different distances from the center of root development are different. Greater amounts of nitrates occur at a distance from the absorption system of the plant.

It seems unlikely that the modification of the nitrate content by plant development is a factor of importance in bringing about the acceleration in microbial activity which has been observed to accompany plant growth.

The results are discussed in regard to the influence of plant development upon variation in nitrate content of soils and the relationships between nitrate content, soil fertility, and microbial activity.

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Handbuch der Pflanzenernährung und Düngerlehre, vol. I. Edited by F. HONCAMP. Julius Springer, Berlin, 1931. Pp. xv + 945, figs. 90.

This is the first volume of the Handbuch, devoted to plant nutrition. In preparing this volume, the editor had the assistance of noted authorities in their several fields. The major topics dealt with by the authors include a historical review of the evolution of our knowledge of plant nutrition; the proximate and ultimate composition of plant substances; the cycle of plant nutrients; the physiology of plant metabolism; the soil as a site and reservoir of plant nutrients; the so-called laws of crop yields; water culture and pot experiments; field experiments; the interpretation of fertilizer experiments; and the determination of the plant food requirements of soils. As indicated by this outline, the authors have made a very ambitious effort to review rather exhaustively the field of plant nutrition. The plant physiologist, the student of soil science, the agronomist, and the agricultural chemist will find in this work a wealth of information systematically arranged and discussed. The very extensive bibliography covers most thoroughly the writings of German investigators in the field of plant nutrition. At the same time, the references to the corresponding scientific literature of other countries are almost as complete.

Chemistry for Students of Agriculture and Home Economics, First Edition. By ROBIN CHARLES BURRELL. McGraw-Hill Book Company, Inc., New York and London, 1931. Pp. xviii + 459, figs. 77.

Chemistry is playing an increasingly important part in the training of students in agriculture and allied subjects. Soils and their management, farm manures and amendments, and particularly commercial fertilizers, represent materials whose chemistry is now better understood. Insecticides and fungicides, preservatives and antiseptics, plants and their derivatives, and animals and their derivatives are economically significant largely because of their chemical make-up. It is obvious, for this reason, that the author was justified in stating in the preface that "training in chemistry for students who do not intend to become specialists in this field of knowledge, must be designed simply to lay a broad general foundation. Many of the individual specific facts of the chemistry of tomorrow will be quite different from those of the chemistry of today. Yet these students as intelligent, wide-awake citizens, taking an active part in the world's work, will want to be able to understand the most recent discoveries about plant growth, the chemical control of insect pests and plant diseases, and the scientific feeding of animals and human beings. They

will want to be able to appreciate the importance of chemistry in industry and the world problems that chemical discoveries often create."

The principal topics dealt with in the book include: fundamental principles of general chemistry; analytical and synthetic chemistry; organic chemistry; biological chemistry; and chemistry and the world's work. Under the topic "Biological Chemistry" the author deals with the fats and related substances, the carbohydrates, the proteins, the chemical and physical nature of living matter, the chemistry of plants, and the chemistry of animals. Under the topic "Chemistry and the World's Work" there is a discussion of chemistry in the home, chemistry in agriculture, chemistry in industry, and chemistry in problems of social welfare. There are five appendices, a glossary, and an adequate index.

The Soil and the Microbe. By SELMAN A. WAKSMAN and ROBERT L. STARKEY. John Wiley & Sons, Inc., New York, 1931. Pp. xi + 260, figs. 85.

The authors have appropriately dedicated their book to Sir John Russell, Director of the Rothamsted Experimental Station in England and widely known authority on plant nutrition, soil chemistry, and soil microbiology.

A conception as to the contents of the book may be had from the authors' statement in the preface. They say: "Our knowledge of the soil microbes and their rôle in soil processes and plant growth has developed in the last fifty years. However, a large body of information has since accumulated which enables us to construct a clear picture not only of the microscopic population of the soil, of its numerous physiological reactions, but also of the relation of these processes to the origin and formation of soil, to the cycle of elements in nature and to plant nutrition." The major topics dealt with by the authors consist of the soil and the plant, the microbe and its activities; the soil population and its distribution; the rôle of microbes in the distribution of organic substances in the soil; the transformation of nitrogen by soil microbes; the transformation of mineral substances in the soil through direct or indirect action of microorganisms; the interrelation between higher plants and soil microorganisms; modification of the soil population; and importance of microbes in soil fertility.

The undergraduate as well as the advanced student will find in this book a summary of pertinent facts on the subject of soil microbiology. The authors' intimate knowledge of the field assures us of the accuracy of the statements made by them and of the reasonable completeness of the information given by them. The book should stimulate an interest in an important part of the broad field of soil science and should offer to the instructor a textbook of the type not hitherto available.

Soil Management, Second Edition. By FIRMAN E. BEAR. John Wiley & Sons, Inc., New York, 1931. Pp. v + 412, figs. 58.

The purpose of the author in writing, and later revising, his book is well

indicated in the preface to the second edition. He says: "The purpose of this book is primarily that of acquainting the student with the applications of those scientific facts and principles that are of use in planning constructive systems of soil management and increasing the productive capacity of soils." The 26 chapters of the book are grouped under five larger divisions, designated, respectively, as Requirements of Crops; Characteristics of Soils; Utilizing Soil Resources; Conserving Soil Resources; and Supplementing Soil Resources.

While it is obvious that it is not possible to offer an exhaustive treatment of the subject within the scope of 400 pages, the author has been successful, nevertheless, in effectively organizing a large mass of facts and deductions and of presenting it in very readable form. The revised edition should prove as widely useful as is the first edition to beginners as well as to fairly advanced students of soils and soil fertility. The book will also be found to have a distinct value for reference purposes.

J. G. LIPMAN.